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THE PNEUMONITIS AND GRANULOMATOSIS PECULIAR TO BERYLLIUM WORKERS *

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The pathologic aspects of the acute pneumonitis and chronic pulmonary granulomatosis of beryllium workers have been discussed briefly in several publications.[1-4] There has been no previous attempt to describe the changes in the tissues of groups of cases representing both acute pneumonitis and granulomatosis. This report has been compiled following a study of tissues from the bodies of 7 persons who died of acute pneumonitis and of 13 persons who died of chronic granulomatosis.

BERYLLIUM AS A PATHOGENIC AGENT

There is evidence that beryllium is not an innocuous substance when in contact with living tissues. This has been recognized in beryllium-using industries for several years, in which it was observed that the accidental introduction of beryllium compounds beneath the skin resulted in chronic ulcers which persisted until the tissues containing the metal were excised or until the foreign material was eliminated. Local tissues from 3 cases in which beryllium phosphors † had been accidentally introduced beneath the skin were examined in this laboratory. The similarity of the lesions in these subcutaneous sites to each other, and to the granulomas which were found in the lungs of persons who died of beryllium granulomatosis, was so marked as to provide fortuitous but highly advantageous evidence of the pathogenicity of beryllium.

Further evidence that beryllium in the tissues stimulates an inflammatory response has been presented by surgeons who have experimented with metals in repairing bone defects.[5] It was found that an

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† Beryllium phosphors are powders which contain from 4 to 12 per cent beryllium, expressed as beryllium oxide.

alloy containing nickel, cobalt, chromium, and molybdenum produced no tissue reaction, but that the addition of 1.6 per cent of beryllium to the alloy caused the formation of "chronically inflamed granulation tissue containing many macrophages." It was also reported that there was extensive fibrosis with marked lymphocytic infiltration.

Beryllium Pneumonitis and Beryllium Pneumonoconiosis

The clinical aspects of the pulmonary disease peculiar to beryllium workers have caused it to be classified into two mutually exclusive conditions—acute pneumonitis and chronic pulmonary fibrosis—with little clinical evidence of a direct relationship between the two conditions. Acute pneumonitis has been marked by a sudden onset, with cough, chest pain, dyspnea, cyanosis, diminished vital capacity, and loss of weight. The illness lasts from 5 weeks to 4 months and the mortality rate is about 1 per cent. Pulmonary fibrosis (delayed pneumonitis,[2] chronic pulmonary granulomatosis[6]) has had an insidious onset during which there is increasing weight loss and weakness, cough, and dyspnea. Some of the patients with chronic granulomatosis are alive and working 5 years after the diagnosis was made. About 10 per cent of all patients diagnosed have died. From the standpoint of morbid anatomy this differentiation into acute and chronic diseases is not clear-cut, and all stages of transition have been seen between the conditions designated as acute pneumonitis and as chronic granulomatosis.*

Furthermore, the designation "acute pneumonitis" is inadequate from the pathologic standpoint in some cases which fit clinically into this category, because organization of exudate, which is not ordinarily considered as part of a strictly acute reaction, has been noted in 6 of the 7 cases of acute pneumonitis from which I have had tissues. Similarly, the designation " chronic granulomatosis" is likewise inadequate, because it neglects other important pathologic aspects of the clinically chronic disease. Emphysema and diffuse fibrosis are presumably much more important, from the standpoint of the well-being and pulmonary function of the patient, than are the granulomas.

The development of granulomas has been studied through a number of the transitions in the lungs of various patients. The earliest phase

* After this paper was submitted, it was brought to my attention that at the Greater New York Safety Conference, April 13–16, 1948, Dr. Willard Machle stated that "the differentiation between acute, subacute, and chronic disease from beryllium is purely arbitrary. Our own experience indicates that all gradations in rate of onset, severity, and resolution on a time base may be seen." The differences, Dr. Machle pointed out, are probably related to the solubility of the compounds to which the patients were exposed.

of a recognizable granuloma has been seen in a patient who died of acute beryllium pneumonitis. The well developed granulomas have all been confined to patients who died of chronic granulomatosis.

Tissues from 10 patients who died of beryllium pneumonitis or granulomatosis have been analyzed for beryllium by the method of Cholak and Hubbard.[7]

Table I is presented as a guide to the cases which form the basis of this report upon the pathologic aspects of pulmonary disease of beryllium workers. The results of analyses of the lung tissues are included also.

GROSS MORPHOLOGIC FINDINGS

I have made gross examinations of tissues from 5 persons who died of acute pneumonitis of beryllium workers. In these cases the basic pulmonary lesions have been similar. The lungs have been heavy, the combined weight of both lungs being more than 2,000 gm. in 2 of the 5 cases. The lungs had lost their usual elasticity, so that when the chest cavities were opened they retained their expanded shapes. The cut surfaces were fairly homogeneous, and ranged from pinkish gray to bluish gray. Moderate quantities of thin fluid could be expressed. There was little fluid in the bronchi and bronchioles, and no pus was seen. In each case there was moderate enlargement of the bronchopulmonary and peritracheal lymph nodes, and these were soft and fleshy. In one case there was a partially organized infarct in the lower lobe of the right lung, with a thrombus in an artery at the apex of the infarct. In another case the heart was somewhat flabby and slightly dilated. In all cases of acute pneumonitis, there were hyperemia and parenchymatous degeneration of the viscera.

In the cases of chronic beryllium granulomatosis, also, the lungs have been heavy, usually ranging between 1,500 and 2,000 gm. In one case they weighed 2,400 gm. Although the lungs had lost their elasticity and remained in the expanded position, they did not have the homogeneous character of the lungs in cases of acute pneumonitis. In chronic granulomatosis the lungs contained many small, firm nodules, ranging up to 2 mm. in diameter. Emphysema was present invariably, and in most cases the nodules and enlarged air spaces were scattered diffusely throughout the lung. In a few cases the emphysematous areas were limited to focal regions up to 5 or 6 cm. in diameter, which alternated with masses of dense tissue of similar or larger size. Some lungs had a generalized honeycombed appearance due to emphysema, and occasionally there were subpleural emphysematous blebs. In one case a small hemorrhagic infarct was seen in the lower lobe of the right lung. There were focal fibrous adhesions between the visceral

TABLE I

Cases of Pulmonary Disease in Beryllium Workers

Case number	Clinical type	Duration of illness	Approximate length of exposure	Type of exposure	Micrograms of beryllium in 100 gm. of lung tissue	Characteristics of lesions
H-1	Acute	14 days	40 days	Alloys (BeO)	*	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization
D-1	Acute	16 days	6 yrs.	Ore reduction	220	Acute pneumonitis; edema; mononuclear, plasma cell, lymphocytic exudate; no organization
H-2	Acute	21 days	49 days	Ore reduction	128	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization
C-2	Acute	21 days	90 days	Phosphor	20	Acute pneumonitis; mononuclear cell and lymphocytic exudate; organization of exudate; early granulomas
D-2	Acute	27 days	11 mos.	Ore reduction	9	Acute pneumonitis; mononuclear cells, plasma cells, lymphocytes; early organization and beginning of nodule formation
D-3	Acute	14 days	30 days	Ore reduction	12	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization and granulomas
H-3	Acute	61 days	9 mos.	Alloys (BeO)	*	Mononuclear, plasma cell, lymphocytic exudate; early organization
A-1	Chronic	17 mos.	13.5 mos.	Phosphor	*	Fibrosis, granulomas, slight emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
A-3	Chronic	7 mos.	9 mos.	Phosphor	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; no conchoidal bodies
B-1	Chronic	24 mos.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies

Table I (Continued)

Case number	Clinical type	Duration of illness	Approximate length of exposure	Type of exposure	Micrograms of beryllium in 100 gm. of lung tissue	Characteristics of lesions
B-2	Chronic (accidental death)	28 mos.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-3	Chronic		6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-4	Chronic	2 yrs.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-5	Chronic		6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
B-6	Chronic	5 yrs.	43 mos.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
C-1	Chronic	13 mos.	4 yrs.	BeO	12.0	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies; some nodules completely fibrotic
E-1	Chronic	3 yrs.	?	BeO	1.7	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies; some nodules completely fibrotic
F-1	Chronic	1 yr.	4 yrs.	BeO	78	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies
G-4	Chronic	15 mos.	5 yrs.	Neighborhood of ore reduction plant	0	Fibrosis, granulomas, emphysema; lymphocytic and mononuclear exudate; conchoidal bodies
G-5	Chronic	18 mos.	1 yr.	Neighborhood of ore reduction plant	0.93	Fibrosis, granulomas, emphysema; lymphocytic and mononuclear exudate; no conchoidal bodies

*No analysis made.

and parietal pleurae in 5 cases. The peribronchial and peritracheal lymph nodes were moderately firm and enlarged in nearly all cases.

In every case of chronic beryllium granulomatosis there was thickening of the wall of the right ventricle of the heart, with dilatation of this chamber. In most cases this was slight but in some it was marked and a true cor pulmonale was present. In addition, there were evidences of chronic passive hyperemia of the abdominal viscera in these cases. There were no renal calculi in the cases reported here, but it is probably significant that such calculi from 2 persons with chronic granulomatosis have been analyzed at the Kettering Laboratory and were found to contain beryllium.

HISTOPATHOLOGIC FINDINGS

Lungs

The lungs in the 7 cases of *acute pneumonitis* of beryllium workers were characterized by inflammatory exudate and the early formation of new connective tissue.

In the earliest phase of the disease, the exudate was comprised of fluid and cells. In some regions, the fluid was seen as homogeneous eosinophilic material, but in other places considerable quantities of fibrin were present also. This fluid filled the lumina of some alveoli, while other alveoli contained relatively small amounts of it. Large mononuclear cells were predominant in the exudate in all acute cases, and among them were scattered lymphocytes and plasma cells in moderate numbers (Fig. 1). These cells, together with a few polymorphonuclear leukocytes and occasional erythrocytes, comprised the only formed elements within the alveolar lumina. The large mononuclear cells had pale reticulated nuclei and pink, finely granular cytoplasm. Many of them had fine granules or fragments of disintegrating cells in their cytoplasm. In the cases of shorter duration, only a few of these cells were vacuolated, but in cases with a duration of 21 days or more, large numbers of the mononuclear cells contained clear vacuoles, so that the cytoplasm appeared foamy. These vacuoles contained lipid material which stained bright orange with sudan IV. The cells lining the alveoli were swollen and some contained mitotic figures. Transition of the lining (septal) cells to free mononuclear clasmatocytes was well represented in all cases of acute pneumonitis.

There were only rare polymorphonuclear leukocytes in the exudate in 6 of the 7 cases of acute pneumonitis. In one case there was a recent pulmonary infarct in which polymorphonuclear leukocytes and erythrocytes were numerous; elsewhere the exudate in this case was like that of the other 6 cases.

In the lumina of some alveoli, there were masses of degenerating

large mononuclear cells. The cells lining these alveoli were invariably enlarged, and in various stages of desquamation. Often there was coalescence of cytoplasm of the desquamated cells to form giant cells around degenerating and necrotic débris. In later stages of this process, there was proliferation of fibroblasts within adjacent alveolar walls, with organization of the periphery of the mass and formation of a granuloma within the lumen of the alveolus (Fig. 3). Silver impregnation revealed early formation of reticulum in the peripheral portions of these masses, with centripetal strands extending toward the centers.

The central débris of the nodules assumed a brightly eosinophilic, homogeneous appearance, characteristic of the ill defined substance or substances called fibrinoid. This fibrinoid substance was stained bright pink in phloxine and methylene blue preparations, green in Masson's trichrome, yellow in van Gieson's, and did not impregnate with silver. The granular débris from which it seemed to be condensed stained blue with phloxine and methylene blue, and green with Masson's trichrome method.

Although the amount of fibrinoid material was small in the majority of cases of acute pneumonitis, it was found in 6 of the 7 cases. In one case it was abundant, and was usually found in regions where clasmatocytes were numerous. It lay free in the alveolar spaces, and usually had a serpentine appearance, although occasionally it formed irregular strands or globules. Often it was partly or completely surrounded by large clasmatocytes which sometimes were fused to form multinucleated giant cells. Where fibroblasts and lymphocytes formed nodules in the walls of septa, masses of fibrinoid substance were often found associated with them (Fig. 4).

The origin of this fibrinoid material is not known, but in one case there was a suggestion of transitional stages from degenerating large mononuclear cells, through slightly eosinophilic granular débris, to increasing condensation, homogeneity, and eosinophilia, and, finally, to the fully homogeneous and markedly eosinophilic mature hyalin.

Hyaline membranes lining occasional alveoli and respiratory bronchioles were found in 2 cases of acute pneumonitis. They were affected by stains in the same way as the fibrinoid material, and presumably represented a vagary of distribution of that substance.

In all cases there was infiltration of the interstitial tissues of the septal walls by lymphocytes and plasma cells. These were relatively few in cases of shorter duration, and were numerous in the lungs of those who survived longer, in which they occasionally were grouped with fibroblasts and reticulum into nodules. Similar cells were present in corresponding numbers in the peritruncal connective tissue.

Fibrosis occurred fairly early in acute pneumonitis. This was mani-

fested by early appearance of reticulum in the intra-alveolar exudate and the growth of fibroblasts into that exudate (Fig. 6). Diffuse proliferation of fibroblasts within the walls of the septa was seen also, and in 3 of the 7 cases of acute pneumonitis there was actual formation of nodules here and there within the septa. These nodules consisted of fibroblasts, lymphocytes, and plasma cells, with eosinophilic granular débris or fibrinoid masses at their centers. In one case in which the duration of the disease was 14 days, well defined granulomas were fairly numerous, both within the alveolar lumina and within their walls.

The bronchi in the cases of acute pneumonitis had intact epithelial surfaces, and their walls were invaded by a few lymphocytes and plasma cells. The respiratory bronchioles of most cases showed infiltration of their walls by large numbers of lymphocytes and desquamation of their cuboidal lining cells.

It appeared that the pulmonary lesions in the 13 cases of *chronic granulomatosis* of beryllium workers were closely related to those in acute pneumonitis. In fact, from the morphologic standpoint, the cases of chronic granulomatosis represented a further development of the acute pneumonitis. Cellular exudate, so prominent in acute pneumonitis, was also a part of granulomatosis. Unlike the situation in acute pneumonitis, however, intraseptal lymphocytes and plasma cells were much more numerous than were cells free in the alveolar spaces. There were large mononuclear cells within the alveoli in all of the chronic cases, the numbers varying greatly from case to case and in different portions of the lungs in a given case. Polymorphonuclear leukocytes were virtually absent from the lungs, except in one case.* In addition, the lungs of the patients with granulomatosis also had numerous giant cells which are described in detail below.

Extensive regions of emphysema were present in all cases, with attenuation of septal walls and enlargement of air spaces. The capillaries of the septa in the emphysematous regions were narrowed, so that this condition, together with fibrosis and granulomas in other septa and in the peritruncal tissues, tended to impede the pulmonary circulation.

There was marked fibrosis of the lung in all instances of chronic granulomatosis. This fibrosis was distributed in the septa, in the granulomas, and in the perivascular and peribronchial regions. The collagen was relatively dense, and only moderate numbers of fibroblasts were visible. There was an increase in the diameter of the

* One patient with granulomatosis, who died with superimposed acute pneumonia, had many polymorphonuclear leukocytes in his lungs.

capillaries of most septa, so that the enlargement of the septa resulted from both dilatation of the vascular channels and proliferation of connective tissue.

The granulomas appeared to be characteristic of the pulmonary granulomatosis of beryllium workers. In the cases of long-standing chronic disease, they were confined to the enlarged septal walls or to the peritruncal connective tissues. In all cases, there were regions in the lungs where overgrowth of fibrous tissue effaced the normal pulmonary structure, forming fibrous zones which were at least partially comprised of old granulomas. Doubtless, they also received contributions from the newly formed collagen of the alveolar walls and peritruncal zones. Within these fibrous areas, granulomas were often close together, and occasionally two were found to be contiguous and merging (Fig. 8).

The granulomas consisted of a peripheral zone of loose fibrous tissue which surrounded a central region comprised of necrotic, granular, eosinophilic débris, of fibrinoid material, or of a Langhans' giant cell. The central regions and the fibrous zones were usually infiltrated with lymphocytes, and moderate numbers of large mononuclear cells often were found in the fibrous zone. These cells were fibroblasts, and they had oval, reticulated nuclei. They were arranged with their long axes circumferential. Cells of epithelioid type, so prominent in Boeck's sarcoid, were relatively infrequent. Within some granulomas, there were peculiar basophilic structures which were identified as the conchoidal bodies often found in lesions of Boeck's sarcoid (Fig. 9). Many of the granulomas had centers which contained none of the aforementioned materials, but were comprised of slightly eosinophilic, relatively acellular, fibrous tissue. Silver impregnation of granulomas of this type revealed that the argentophilic material had a radial arrangement, suggesting an ingrowth of reticulum toward the center from the outer zones.

The giant cells sometimes were found in the centers of the granulomas, but often they were among the peripheral fibroblasts. Frequently, several were scattered about within one granuloma. These cells were quite like Langhans' giant cells, differing from the foreign body giant cells of acute pneumonitis. The nuclei of these Langhans' cells were mostly reticular, but many were pyknotic. Sometimes the nuclei were polar, sometimes circumferential, and occasionally they were scattered without pattern in a local area in the cell. The number of nuclei in cross sections of giant cells varied from 4 to more than 30. Many of these cells were in various stages of degeneration. Most had

brightly eosinophilic, slightly granular cytoplasm, but many had foamy or vacuolated centers. In 2 cases, there were rare asteroid inclusion bodies similar to those described by Wolbach.[8]

Associated with the granulomas, sometimes enclosed in the cytoplasm of a Langhans' giant cell, were the conchoidal bodies to which reference has been made above. They varied from 20 to more than 100 μ in greatest diameter, and were usually roughly ovoid. Those within giant cells sometimes were so large that only a thin rim of cytoplasm and nuclei surrounded them. Those which were not in giant cells were invariably lying either within fibrous tissue or granular eosinophilic débris, and they had the appearance of having outgrown an enveloping giant cell. This has been seen by Schaumann,[9] who described the larger ones as lying in débris of giant cells which had "burst." In some cases these bodies were so numerous that nearly every low-power microscopic field had at least one in it. In other cases a few were found only after extensive search. These bodies were seen in 11 of the 13 cases of chronic granulomatosis. The conchoidal bodies are believed to be identical with those of Boeck's sarcoid. They were blue to black in hematoxylin and eosin preparations, red in van Gieson's preparations, and gave a strongly positive reaction for iron. They were not impregnated with silver salts by Laidlaw's method. Their origin is unknown, and, as pointed out by Schaumann, has been the subject of considerable controversy.

The final stage of the granuloma was seen in sections from 5 cases. This was a single nodule, or a coalescence of several nodules, which had become completely fibrotic (Figs. 10 to 13). Superficially, these resembled the nodules of silicosis, but instead of the tissue being more or less regularly whorled, the strands tended to take a circumferential direction. These fibrous nodules showed relatively little hyalinization, as compared to silicosis, and the individual fibrils were fairly well distinguished. Finally, silver impregnation in these cases failed to reveal central, deeply argentophilic material of the type described by Belt.[10]

Microscopic examinations of tissues from persons who died of granulomatosis, utilizing ultraviolet illumination, have revealed fluorescent granules in the lungs of some cases. These studies will form the basis of a subsequent report. The lungs of all cases were examined for acid-fast organisms, and none were found.

Alterations of the pulmonary blood vessels included perivascular fibrosis and nodules encroaching on the adventitia in all cases. In one case there were also numerous thrombi in the smaller tributaries of the pulmonary veins, and these showed advanced organization. The intimal

tunics of the larger arteries were thickened in all cases by the presence of large foam cells of the type common in atherosclerosis. This was due presumably to hypertension of the lesser circulation as a result of the narrowing of the capillary beds.

Bronchopulmonary and Mediastinal Lymph Nodes

The bronchopulmonary and mediastinal lymph nodes in cases of *acute pneumonitis* were hyperemic, and their sinusoids were filled or partially filled by large mononuclear cells. These cells resembled those which comprised most of the pulmonary exudate. They were actively phagocytic, as evidenced by the fact that the cytoplasm of many enveloped débris of disintegrated cells, whole lymphocytes, or partially disintegrated erythrocytes. The majority of these cells had foamy cytoplasm. The follicles showed relatively little change in most instances, but in one case their centers were in various stages of degeneration. These changes ranged from disappearance of single cells or pyknosis of nuclei to complete necrosis of the central regions with conversion of the centers to eosinophilic granular débris. The lymph nodes of 2 patients had small hyalinized masses of connective tissue, which were relatively acellular and presumably represented old, healed, tuberculous lesions.

The bronchopulmonary and mediastinal lymph nodes in *chronic granulomatosis* showed changes which reflected those in the lungs. These were fibrosis, granulomas, and giant cells. In those cases in which conchoidal bodies were present in the lungs, they were found also in the lymph nodes. In the nodes of several patients there were masses of connective tissue which resembled healed tubercles.

Lesions in Other Viscera

The other viscera in the group representative of *acute pneumonitis* were normal, with one exception. The latter individual had marked centrolobular necrosis of the liver, which was so extensive in most areas that the middle zones also were necrotic. The necrotic regions retained the general architectural arrangement of the liver, the parenchymal cells having been converted to granular eosinophilic ghosts. There were numerous leukocytes of the polymorphonuclear type infiltrating many of the necrotic regions, while other lobules with necrotic centers had no infiltrating cells (Fig. 15). In the same patient there were several focal areas of coagulation necrosis in the vertebral bone marrow. The other organs were normal.

The only lesions outside of the respiratory tract and its lymph nodes, resembling the lesions of *chronic granulomatosis*, were found in

the liver of one patient. These consisted of granulomas within the lobules, consisting of masses of fibrinoid material or of loose connective tissue in which were numerous lymphocytes and plasma cells. Occasional granulomas contained Langhans' giant cells near their centers. There was considerable fibrosis throughout this liver. In some places the connective tissue formed strands which gave the liver the appearance of a cirrhosis of peculiar type. There were polymorphonuclear leukocytes in moderate numbers, as well as lymphocytes and plasma cells, scattered throughout the strands of connective tissue. In one other patient, still alive, a biopsy of the liver revealed similar granulomas, and specimens taken for biopsy of cervical and axillary lymph nodes in several other cases have contained granulomas. In every fatal case, there was thickening of the myocardium of the right ventricle with hypertrophy of the muscle fibers. The livers and other viscera in these cases showed evidences of chronic passive hyperemia.

The So-Called Neighborhood Cases

In the vicinity of industrial plants which are extracting or utilizing beryllium, physicians have become conscious of the hazard to health from fumes or dusts which contain this metal. Pulmonary fibrosis occurring in persons living near a beryllium-handling plant has raised the question of the possibility that beryllium was the cause of the fibrosis. Some such cases have already been shown not to be related to beryllium. Tissues from 2 bodies, concerning which the only exposures had been residence in the neighborhood of a beryllium-reduction plant, were submitted to the Kettering Laboratory for study and analysis. The pulmonary lesions were found to be those of beryllium granulomatosis. In one of these cases an attempt to isolate beryllium from the lungs failed, while in the other case it was possible to isolate 0.93 μg. of beryllium per 100 gm. of lung tissue.

Discussion

There is considerable evidence that the conditions described are the result of the inhalation of fumes or dusts which contain beryllium. The fact that compounds of beryllium are capable, in themselves, of producing inflammation and fibrosis has been demonstrated by work at the Saranac Laboratory,[3,6] as well as by experiments being conducted at the Kettering Laboratory. Lesions identical with those in the lungs of man have not been reproduced in experimental animals, but fibrosis and granulomas which closely resemble human lesions have resulted in the lungs of animals after exposures to beryllium dusts.[6] The constant recovery of beryllium from the tissues of persons who

have died with either acute pneumonitis or chronic granulomatosis further attests the relationship of this metal to the pulmonary conditions. Its relationship to the specific granulomas is apparent in the cases of cutaneous and subcutaneous granulomas which resulted from accidental introduction of phosphors into these tissues.

Since the position of beryllium on the periodic chart of the elements is among metals which are not regarded as disease-producing, beryllium has been suspected of having unique properties to account for its pathogenicity. A number of physicians who are acquainted with the beryllium problem have speculated on the possibility that radioactivity might account for some or all of its harmfulness. I am not sympathetic with this suggestion since repeated examinations of products from which pathogenic fumes and dusts have been derived have yielded no evidences of radioactivity.

The lesions of acute pneumonitis of beryllium workers are not specific for that condition in their earlier phases. The pulmonary edema and mononuclear exudate are similar to the lesions produced by the inhalation of cadmium fumes,[11] and manganese fumes seem to have caused an analogous condition in the lungs of experimental animals.[12] Kneeland and Smetana [13] have described the lungs in a fatal case of atypical pneumonia, wherein they stated that large mononuclear cells formed the most prominent part of the exudate. Furthermore, there was a marked tendency to proliferation of fibroblasts and laying down of collagen in the alveolar walls and in the perivascular regions. The lesions of the lungs in Q fever [14] are also similar to those of the early phases of acute beryllium pneumonitis. In Q fever, the alveoli contain large clasmatocytes and there is infiltration of the alveolar walls by lymphocytes and plasma cells. Acute beryllium pneumonitis can be differentiated from pneumonia due to Friedländer's bacillus, with its exudate of large mononuclear cells, by bacteriologic methods; in the latter, the organisms can be cultured from the lungs, and they are also readily stained in tissue sections. On the other hand, the history of exposure to beryllium and the recovery of this metal from the urine before death or from the lungs removed at autopsy serve to differentiate beryllium pneumonitis from all other conditions.

With the beginning of the development of granulomas, as early as 2 weeks after the onset of acute pneumonitis, the character of the lesions comes to differ from that of any other pathologic process. Transitional stages, from the nodules in acute pneumonitis to well defined specific granulomas in chronic granulomatosis, are clearly presented in the material reported here. The lesions of chronic granulomatosis of beryllium workers resemble to some extent those of several

other more or less well defined conditions. Among these are Boeck's sarcoid,[15] "acute diffuse interstitial fibrosis," [16] and pulmonary fibrosis in a man who worked with radioactive metals.[17]

Beryllium granulomatosis has been confused with Boeck's sarcoid by clinicians, radiologists, and pathologists. Several persons now believed to have beryllium granulomatosis have been considered previously to be suffering from Boeck's sarcoid on the basis of roentgenograms and biopsies of lymph nodes. Differentiation between these conditions can be made readily by microscopic examination of the tissues. The specific granuloma of sarcoid is comprised characteristically of a mass of fairly compact epithelioid cells which usually surround a central giant cell. The giant cells tend to be large, and to have many (usually about 25 or 30) peripheral nuclei. The entire nodule is surrounded by loose connective tissue in which there are nearly always a number of lymphocytes. There is little or no fibrosis or exudate, apart from that actually related to the granulomas. In pulmonary granulomatosis of beryllium workers, on the other hand, the granulomas only rarely have giant cells at their centers, and these giant cells differ from those in Boeck's sarcoid, in that there is great variation in the number of nuclei (from as few as 4 to more than 30). Typical epithelioid cells are seldom a part of the granulomas. Most of the granulomas in the lungs of beryllium workers have either fibrinoid material or granular necrotic eosinophilic débris at their centers. The granulomas in beryllium disease are infiltrated with moderate numbers of lymphocytes which are found even within the granular central débris. Furthermore, in beryllium disease, there is diffuse intraseptal fibrosis of a type never seen in Boeck's sarcoid. The large mononuclear cells found in the alveolar spaces in beryllium granulomatosis are not seen as a part of Boeck's sarcoid. Tissues from 7 cases of typical Boeck's sarcoid have been analyzed for beryllium at the Kettering Laboratory, and this metal was not found in any of them.

The cases of diffuse pulmonary fibrosis described by Hamman and Rich [16] and the case of pulmonary fibrosis described by Belt [17] do have the diffuse fibrosis of the lungs found in beryllium granulomatosis, but in neither of these diseases have granulomas or other nodules been described. Furthermore, the cases of Hamman and Rich differed from those in the beryllium workers in that there was virtually no exudate in the former.

Conclusions

Exposures to dust and fumes containing beryllium have resulted in a number of cases of pulmonary disease. These are of two fairly distinct clinical types: an acute pneumonitis and a protracted pulmonary fibrosis and emphysema.

The pulmonary lesions in these two clinical conditions are specific and there is evidence of transition of the pathologic lesions of the acute condition to those of the chronic one.

In the acute pneumonitis there is a boggy, generalized consolidation of lobes, simulating to some extent the stage of gray hepatization in lobar pneumonia. Microscopically, there is diffuse intra-alveolar exudate composed of large mononuclear phagocytes (septal cells) and edema fluid. Intraseptal lymphocytes and plasma cells are also fairly numerous. In one case centrolobular necrosis of the liver has been seen.

With chronic pulmonary fibrosis (granulomatosis), the lungs have been voluminous and emphysematous. Scattered diffusely throughout the lungs were many fine nodules. Enlargement of the right ventricle of the heart was constantly present in these cases. Microscopic characteristics include diffuse interstitial and nodular fibrosis of the lungs together with a peculiar granulomatous reaction.

The granulomas which are typical of beryllium disease are formed in part within alveolar spaces by the organization of exudate, and in part within the septal and peritruncal connective tissues. They are comprised of central regions of fibrinoid material or granular débris, and the central regions are surrounded by a peripheral zone of fibrosis in which there is an infiltration of lymphocytes and plasma cells. The centers of the granulomas are occasionally occupied by giant cells of the Langhans' type. There are conchoidal bodies in most cases, sometimes within giant cells, sometimes lying free in débris or in fibrous tissue.

Granulomas similar to those of the lungs have been found in the skin and subcutaneous tissues into which beryllium oxide or phosphors have accidentally been introduced. These lesions provide further proof of the pathogenic nature of beryllium.

I wish to express my gratitude for clinical data and for the opportunity to examine tissues to Drs. Morris Carmody, J. M. DeNardi, H. S. Martland, H. T. Karsner, J. B. Hazard, and Willard Machle. The photomicrographs were prepared by Mr. J. B. Homan.

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DESCRIPTION OF PLATES

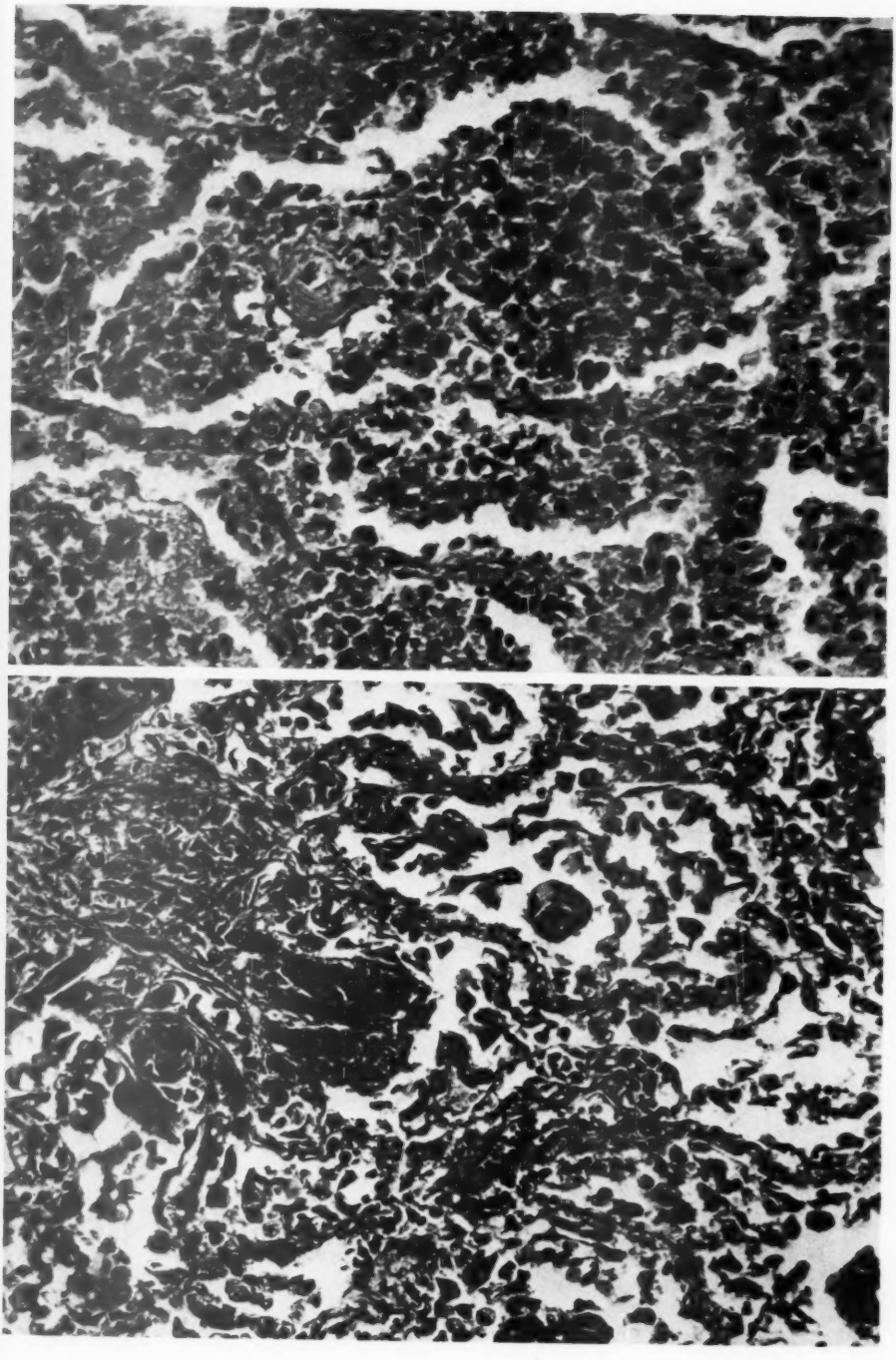
PLATE 185

FIG. 1. Exudate comprised of clasmatocytes, lymphocytes, and plasma cells. Acute pneumonitis (case C-2). × 320.

FIG. 2. Early stage of nodule formation. Septal cells desquamating in sheets in uppermost alveoli. Fibrinoid material in nodules. Acute pneumonitis (case D-3). × 250.

1

2



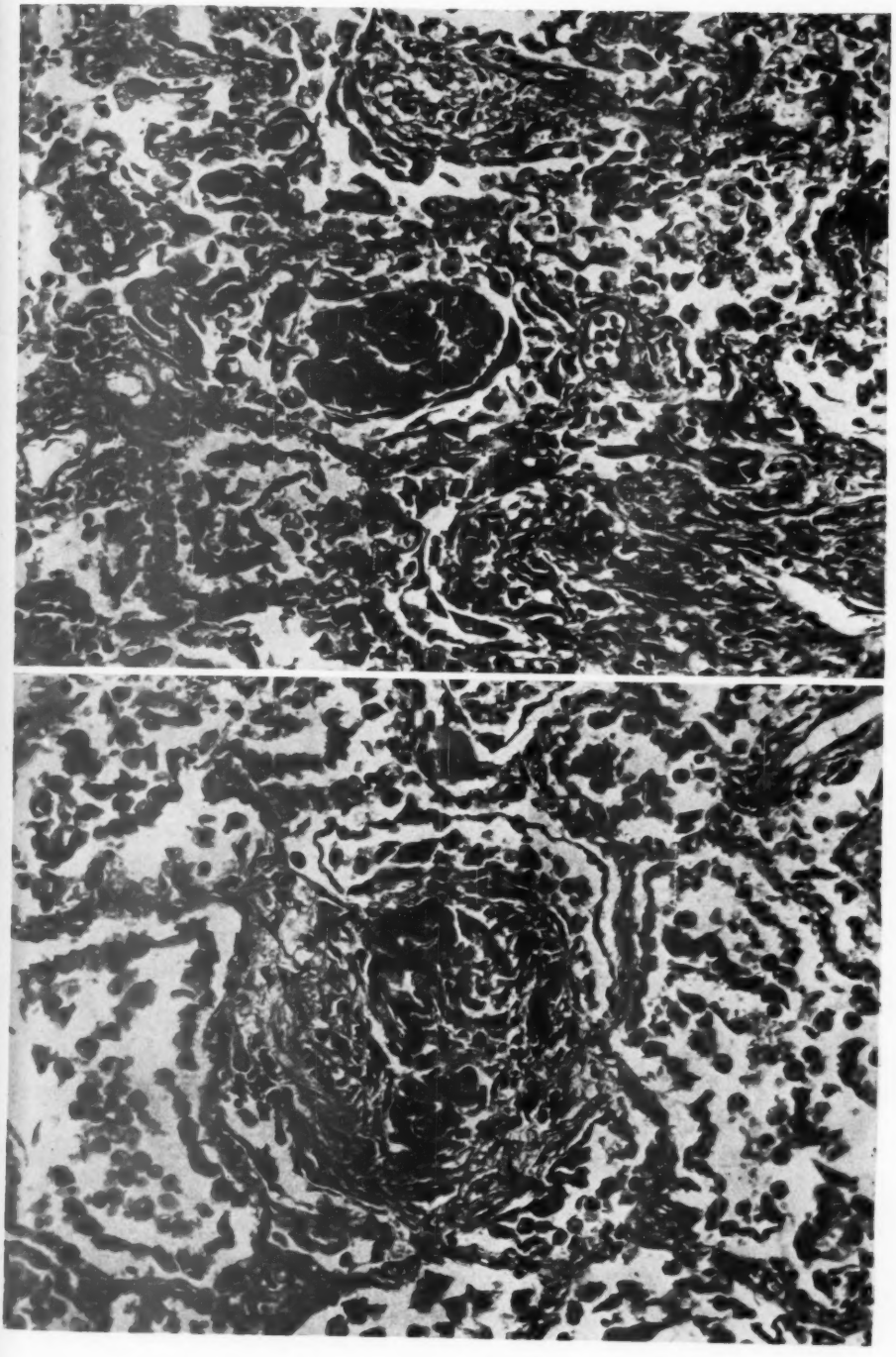
Dutra

Beryllium Pneumonitis

PLATE 186

FIG. 3. Nodule forming within alveolar space. Acute pneumonitis (case D-3). × 250.

FIG. 4. A well formed granuloma in the wall of the septum. Serpentine fibrinoid material is surrounded by loose collagen. Acute pneumonitis (case D-3). × 250.



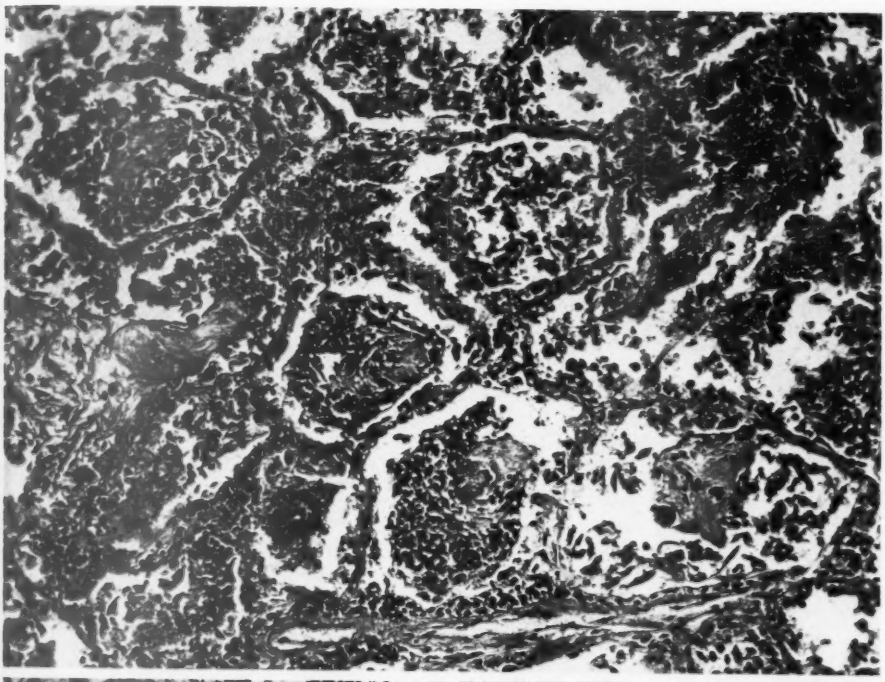
Dutra

Beryllium Pneumonitis

PLATE 187

FIG. 5. Advanced granuloma formation and cellular reaction. Acute pneumonitis (case C-2). × 160.

FIG. 6. Silver preparation showing organization of exudate into granulomas and formation of granulomas in septal walls. Acute pneumonitis (case C-2). × 160.



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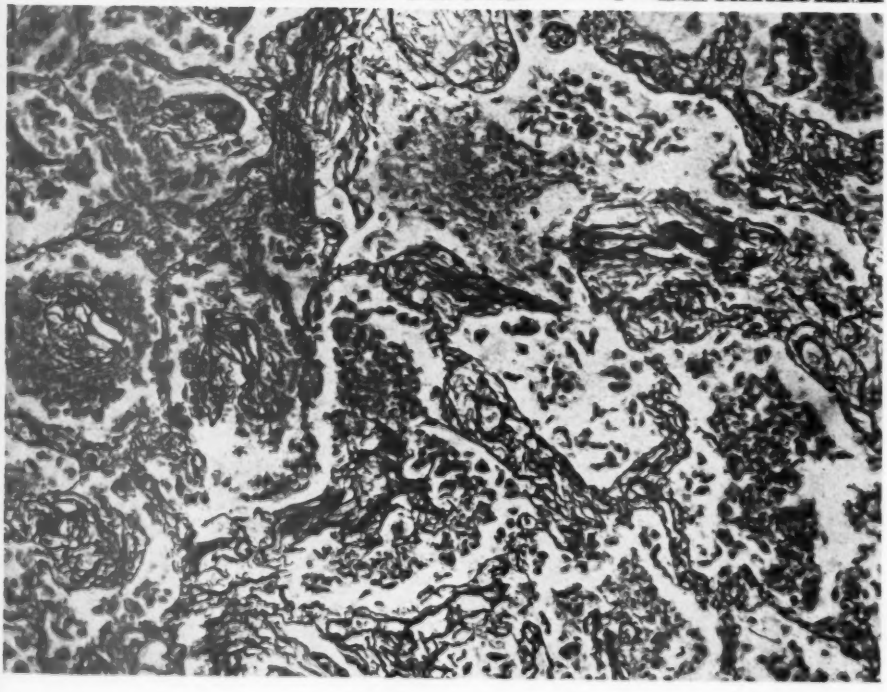

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Dutra Beryllium Pneumonitis

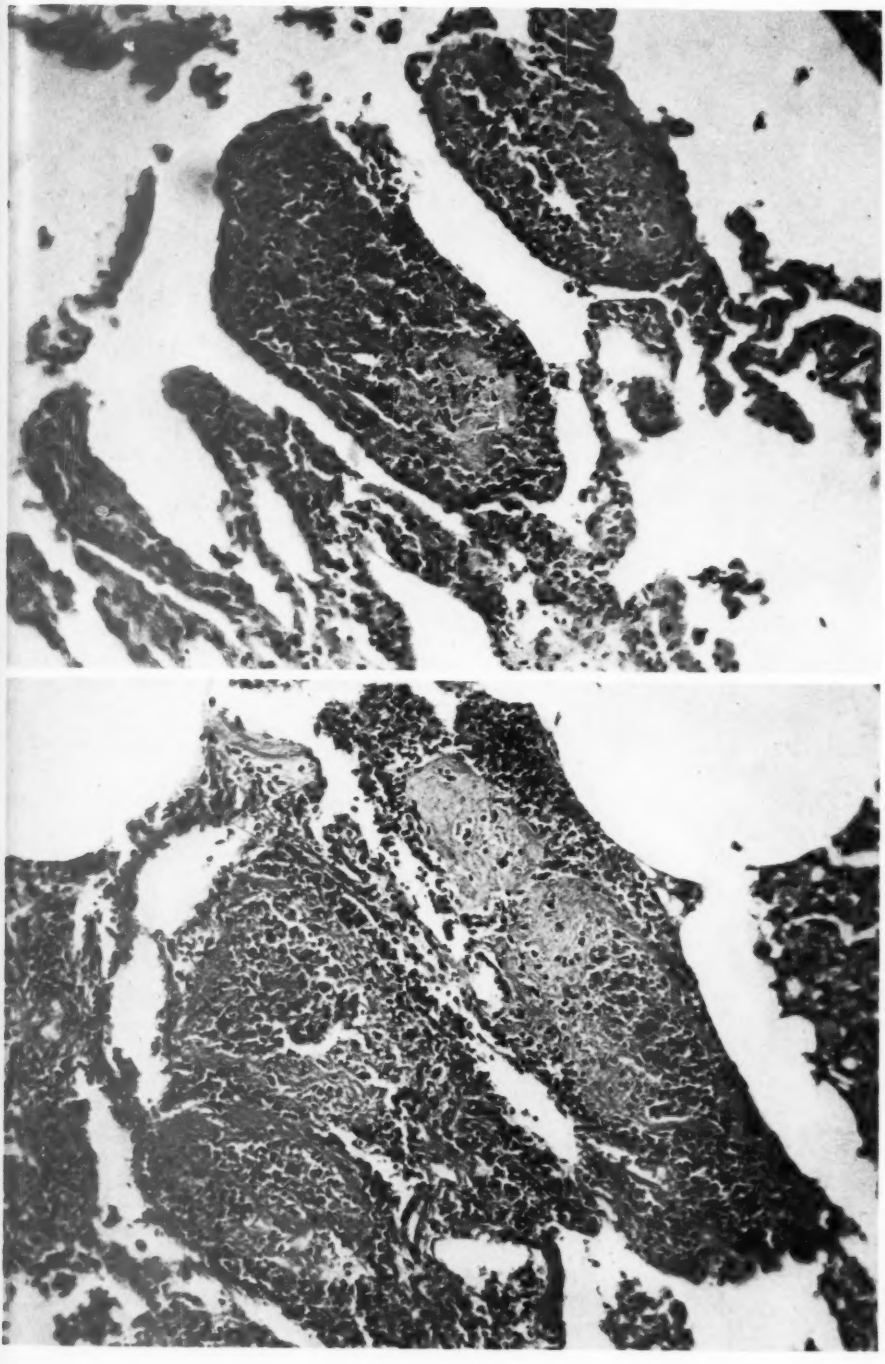
PLATE 188

FIG. 7. Two granulomas in alveolar walls. Chronic granulomatosis (case C-1). × 160.

FIG. 8. Coalescence of granulomas. Chronic granulomatosis (case C-1). × 160.

7

8



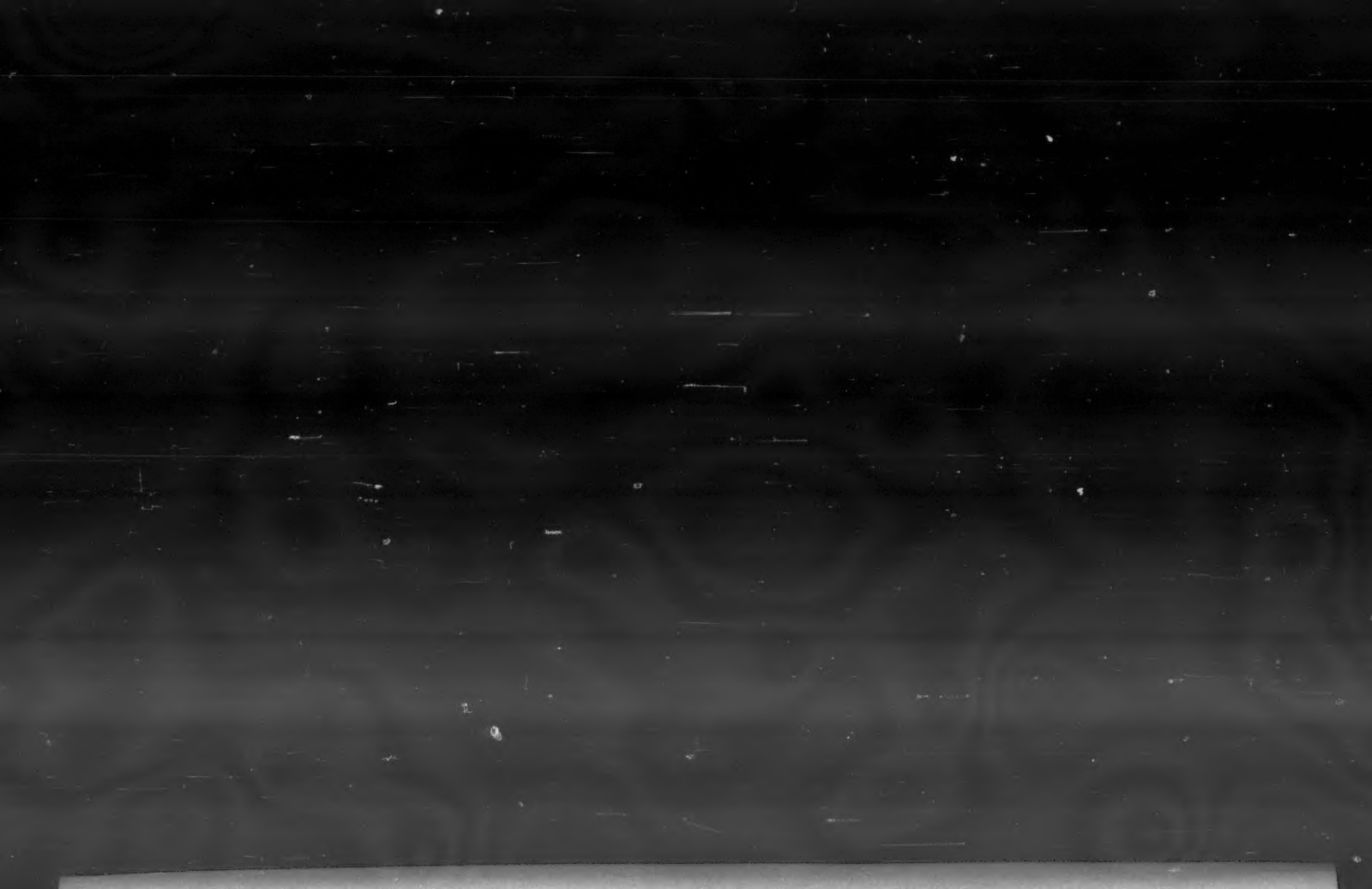
Dutra Beryllium Pneumonitis

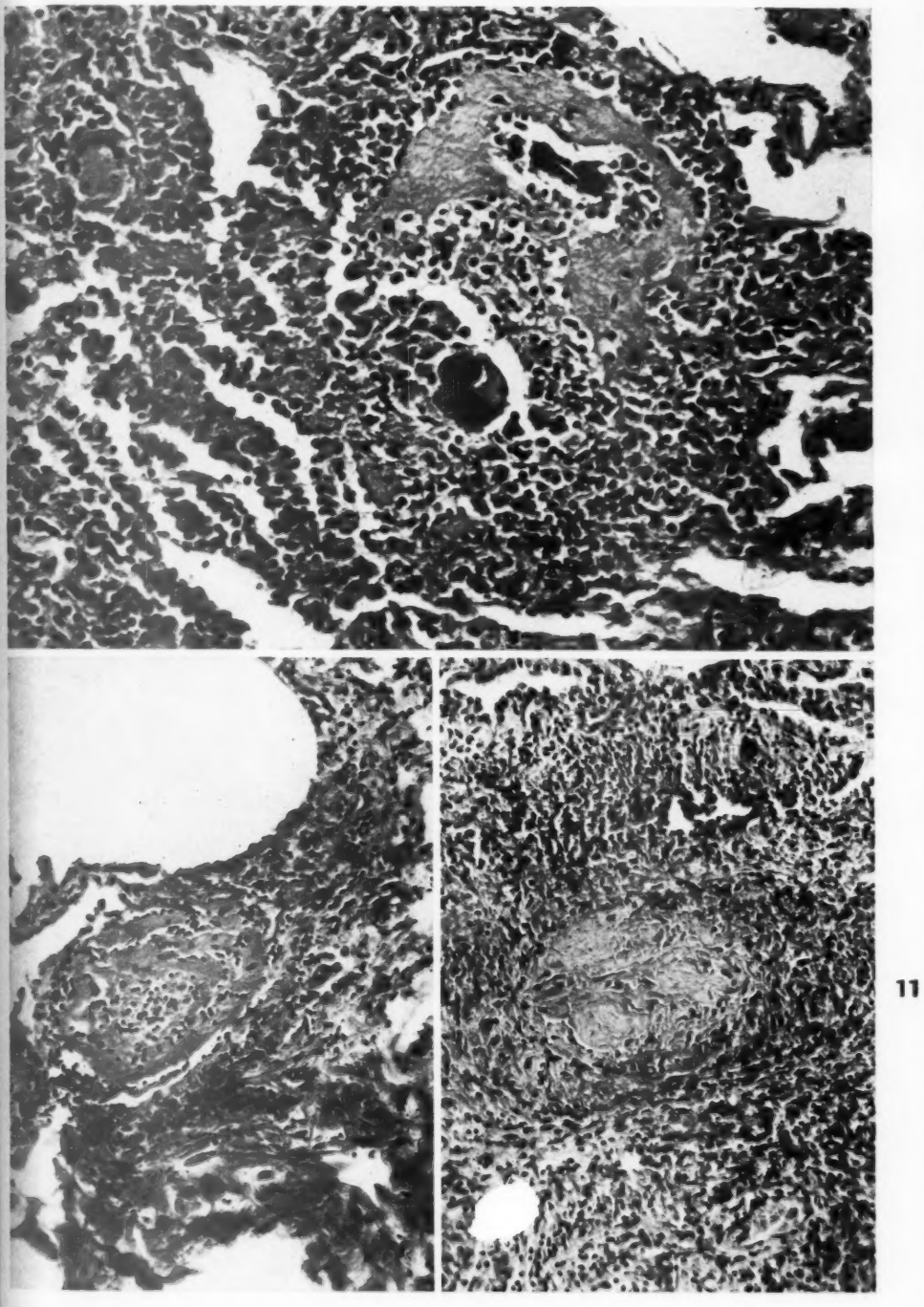
PLATE 189

FIG. 9. Conchoidal bodies. One is within a giant cell and the other lies free in débris within a granuloma. Chronic granulomatosis (case C-1). × 320.

FIG. 10. Fibrosis of a granuloma, early stage, showing thick bands of relatively acellular tissue infiltrated with lymphocytes. Chronic granulomatosis (case C-1). × 160.

FIG. 11. A more advanced stage of fibrosis of a granuloma from a case of chronic granulomatosis (case F-1). × 160.





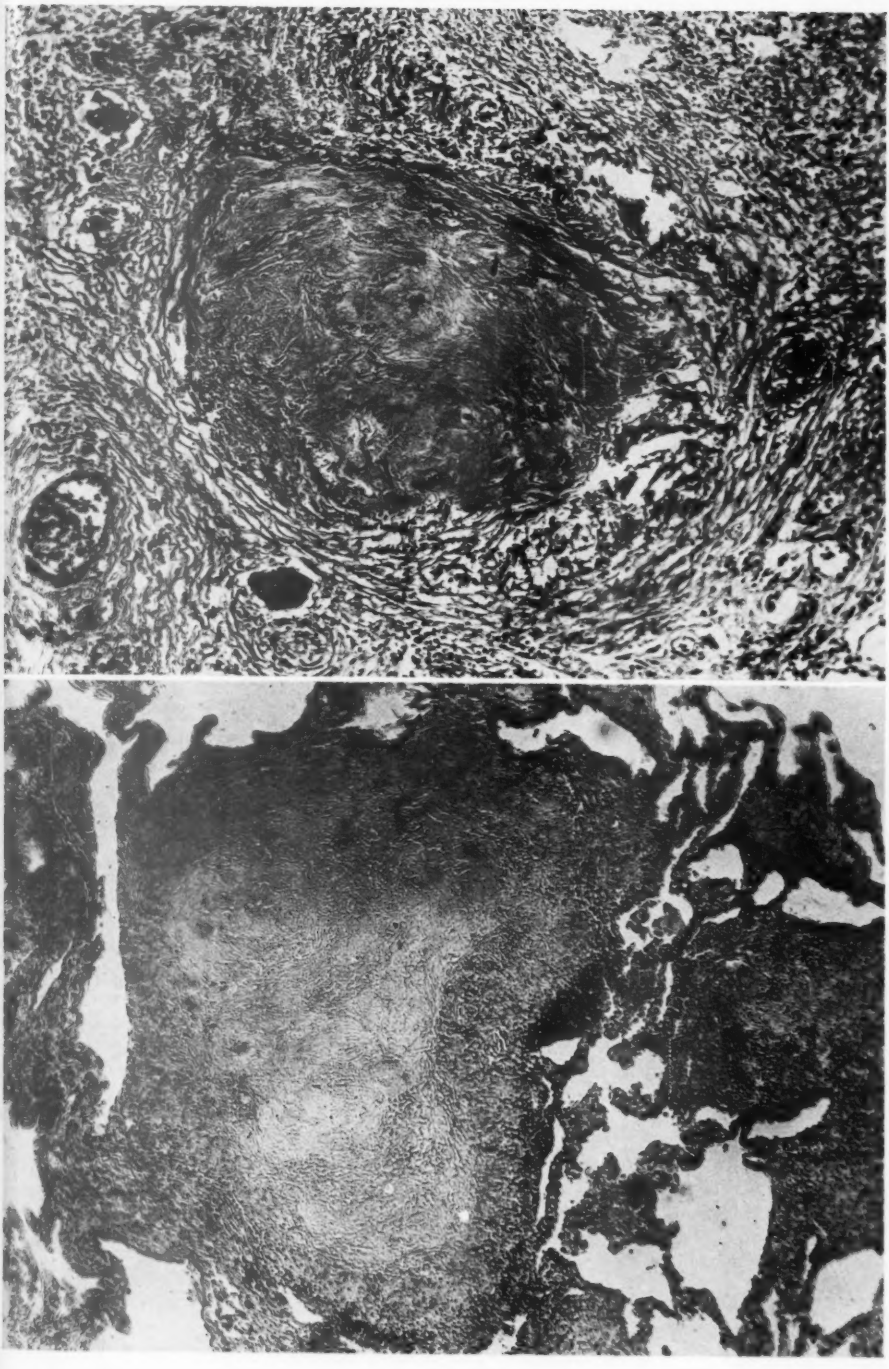
Dutra

Beryllium Pneumonitis

PLATE 190

FIG. 12. A fibrous nodule surrounded by granulation tissue. The absence of whorls and dense hyalinization differentiate this nodule from those of silicosis. Chronic granulomatosis (case E-1). × 125.

FIG. 13. A conglomerate fibrous nodule. Chronic granulomatosis (case A-1). × 85.

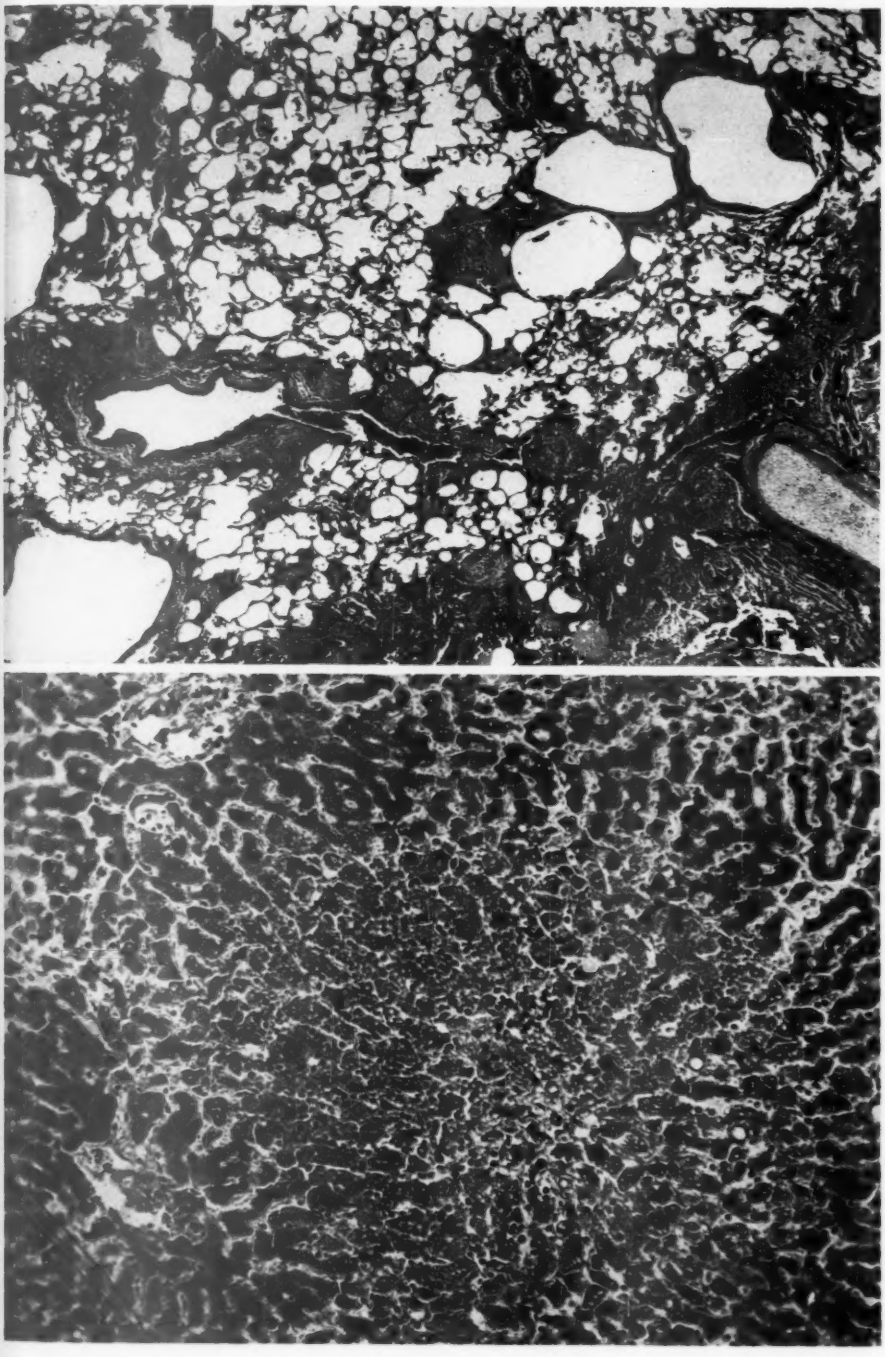


Dutra Beryllium Pneumonitis

PLATE 191

FIG. 14. Low-power photomicrograph to show the locations of nodules in the perivascular tissues and in the walls of septa, and the marked emphysema associated with chronic granulomatosis (case C-1). × 16.

FIG. 15. Liver from a case of acute pneumonitis showing centrolobular necrosis—evidence of systemic toxicity of beryllium (case D-3). × 160.



Dutra Beryllium Pneumonitis

PARAGANGLIOMAS

Review of Subject and Report of Five Original Cases *

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Since Manasse [1] first described an undoubted case of paraganglioma of the suprarenal gland, many interesting facts have come to light regarding these rare tumors. Kohn [2] described the chromaffin system to which these tumors are related. Suzuki [3] first reported a case associated with neurofibromatosis. Labbé, Tinel, and Doumer [4] described the clinical syndrome of paroxysmal hypertension. However, most of the papers dealing with this subject have been isolated case reports with emphasis on some one phase or another. It is our primary purpose to review the literature of this subject, and on the basis of embryogenesis and histology of the paraganglion cells, to evaluate the previously reported cases and to add 5 cases not previously reported.

ORIGIN AND DISTRIBUTION OF PARAGANGLION CELLS

Hollingsworth [5] has recently reviewed the literature concerning the development of the sympathetic system. By a process of maturation and differentiation, ganglion cells of the sympathetic system, paraganglion cells, and the sheath cells (Schwann or neurilemma cells) are derived from the cells of the neural crest. The cells destined to become paraganglion cells migrate to the dorsal surface of the sympathetic ganglia to form small, rounded masses in depressions of these ganglia. Because of this close association, the term *paraganglion* has been applied. Similar masses occur in the sympathetic plexuses, the best known of which are the organs of Zuckerkandl which develop along the aorta near the root of the inferior mesenteric artery. Similar collections of these cells have been described in the liver, testes, kidneys, and heart. The paraganglion cells which form the suprarenal medulla are derived from the celiac plexus; in embryos of 7 weeks, masses of these cells grow into the cortical primordium (from mesoderm) and gain a central position.

Several other tissues have been included in the paraganglionic or chromaffin system, prominent among which are the carotid body, the argentaffin cells of the appendix and intestines, and the coccygeal body. The most dispute has concerned the carotid body. Kohn,[2] studying man and the pig, thought the carotid bodies belonged to the paraganglionic system. He stated that the cells of these tissues are chromaffin cells; that brown granules appear with the use of chromate solutions.

* Received for publication, December 13, 1947.

It is true that the carotid body of pigs contains chromaffin cells, but other workers [6] do not agree that human carotid bodies have chromaffin cells. The carotid body arises from the mesenchyme of the wall of the third branchial cleft and is closely related to the development of a branch of the glossopharyngeal nerve; thus, its origin is different from that of the paraganglion cells. Smith [6] has shown that its connections with the cervical sympathetic nerves are made late in the development of the embryo. She stated that chromaffin cells are not constant in the carotid body of man or the rat, but can be regularly demonstrated in those of the pig and cow. Furthermore, neither epinephrine nor any related vasopressor substance has been demonstrated in the carotid body or tumors derived therefrom. Boyd,[7] in a recent review of the subject, definitely rejected the idea that the carotid body be included in the paraganglionic system. Because of the inconstancy of the presence of chromaffin cells, because epinephrine has never been demonstrated and, most important of all, because of the difference in embryologic development, we will not consider tumors derived from the carotid body as paragangliomas.

There has been less dispute concerning the inclusion of the argentaffin tumors of the appendix and intestine with paragangliomas. Masson,[8] after studying 50 cases of carcinoid of the appendix, confirmed Huebschmann's [9] suggestion that argentaffin cells are chromophilic, but pointed out several reasons why these tumors should not be catalogued with the paragangliomas. First, the argentaffin cells are not derived from the neural crest, but are of entodermal origin. Masson wrote: "All the argentaffin cells enclosed in the nerves result from intranervous budding from the glands of Lieberkühn. The budding epithelia then separate from their gland matrix, migrate into the nerves of the mucosa and differentiate." Further, the argentaffin cells often contain lipoid substances and these are not seen in paraganglion cells. De Castro[10] has shown that the chromaffin reaction sometimes obtained in the carotid body does not depend upon the presence of epinephrine as the reducing substance, but rather on the action of the lipoids contained in these cells. The same might apply to the lipoids of argentaffin cells and the positive chromaffin reaction.

Lewis and Geschickter [11] included argentaffin tumors in their series of paragangliomas, but Geschickter now considers their exact origin to be less certain. For the reasons stated by Masson,[8] in addition to the fact that no epinephrine or similar substance has ever been demonstrated in these tumors, we prefer not to include argentaffin tumors with paragangliomas.

Some early writers have compared the coccygeal body with the

carotid body and so have included it in the paraganglionic system. Because it has nothing in common embryologically with paraganglion cells, we shall not consider it here.

CHROMAFFIN REACTIONS

Bennett,[12] in describing the suprarenal medulla of the cat, presented an excellent survey of the history and the significance of the chromaffin reaction. Henle [13] reported that a dark brown color appeared in the suprarenal medullary cells if they had been subjected to the action of chromic acid or potassium dichromate. Following the identification and synthesis of epinephrine shortly after 1900, it became known that the hormone could be easily oxidized and that the oxidized epinephrine was brown. Hartman and Blatz [14] used the chromaffin reaction to indicate the presence of epinephrine in the suprarenal medulla. Ogata and Ogata [15] thought that the brown color was caused by a precipitate formed by the interaction of epinephrine and chromium compounds. For the following decade the chromaffin reaction was used to indicate the amount of epinephrine in the tissues. In 1930 Gerard, Cordier, and Lison [16] showed clearly that the brown color could be produced by mixing epinephrine with strong oxidizing agents other than chromium compounds. This reaction, then, was not really a demonstration of chromaffinity, but a nonspecific reaction between epinephrine (and related compounds) and strong oxidizing reagents such as solutions of potassium iodate. The term chromaffin reaction is apparently a misnomer since the reaction depends on an oxidation reaction and not on chromium affinity. Organic substances which form these complex compounds with oxidizing agents include epinephrine, hydroquinone, resorcinol, aniline, polyphenols, and many others.

Bennett [12] stated that some of these compounds must be present in all tissues of the body and must account for the light brown color seen in all tissues fixed in dichromate. The much darker color of the suprarenal medulla and paraganglia merely indicates a greater concentration of these fuscogenic substances.

Because the chromaffin test is nonspecific, one should not classify a tumor as a paraganglioma simply because of the presence or absence of a positive reaction. Of greater importance are its origin from cells which have been derived from the neural crest and the presence in it of cells capable of secreting a pressor substance. In a practical sense, then, when one is attempting to classify a tumor suspected of being a paraganglioma he should consider the site of origin and the histologic resemblance to paraganglion cells of the suprarenal medulla. The histologic appearance may vary greatly and may be of little help in

classifying and identifying these tumors. We shall consider as being typical paragangliomas only those tumors which arise in the suprarenal medulla, or in association with the sympathetic ganglia, or in the sympathetic plexuses. The discussion will thus be limited and the statistics presented will be at variance with those by authors who have employed the term in a less restricted fashion.

Report of Cases

Case 1

A Negro housewife, 43 years of age, was first admitted to the Detroit Receiving Hospital on December 31, 1945, because of vaginal bleeding and weakness of 5 days' duration. The positive physical findings included pale mucous membranes, soft cervix with patulent os, and a large nodular uterus. Serologic tests for syphilis were negative and the hemoglobin value was 7.0 gm. per 100 cc. of blood. The clinical impression was complete abortion, multiple leiomyomas of the uterus, and secondary anemia. Treatment consisted of pituitary preparations and multiple blood transfusions. She responded well and was discharged on January 11, 1946, to return to the out-patient department. The blood pressure was not elevated. She was readmitted to the hospital on May 30 in order that definitive treatment for the uterine tumors could be carried out. At this time the blood pressure was 110/70 mm. Hg. The remaining physical findings suggested only multiple leiomyomas of the uterus and secondary anemia. On June 5 supracervical hysterectomy and appendectomy were performed without incident through a midline incision. While exploring the abdomen a retroperitoneal tumor was palpated slightly to the right of the midline and at the level of the third lumbar vertebra. This mass was black, moderately firm, and measured about 6 cm. in maximum diameter. The peritoneum was incised and the mass was found to be attached by a small pedicle to the retroperitoneal tissue. The exact relation to the abdominal aorta was not determined. The postoperative course was uneventful and the patient was discharged on June 17 to the out-patient department. In the succeeding 5 months she was followed at regular intervals and her progress was very satisfactory.

The specimen consisted of a thinly encapsulated, firm neoplasm weighing 30 gm. and measuring 5 by 3 by 2.5 cm. The formalin in which the tumor was immersed was colored brown. Approximately 90 per cent of the tumor was blue-black, there being a small, light-pink area at one end. The demarcation between the pigmented and nonpigmented portions was abrupt. Numerous slit-like cavities were seen in the pigmented portion. The tumor was composed of irregular polygonal cells arranged in solid groups, which were separated one from another by a delicate connective tissue stroma and thin-walled capillaries. In sections prepared from the pigmented areas the neoplastic cells contained a granular pigment, which on low-power examination appeared black and with higher magnification appeared brown-black. Sections from the light areas were almost devoid of pigment. The capillaries, stroma, and alveolar pattern were more prominent in the pigmented areas. The Prussian blue reaction for the presence of

iron was negative. Dopa and chromaffin tests were not done on the fresh material. Staining with the Mallory-Heidenhain technic, as recommended by Gomori,[17] gave negative results. This is an azocarmine stain with which substances related to epinephrine appear as violet granules in the cytoplasm of chromaffin cells. The pigment was darkened by aqueous silver nitrate and bleached with hydrogen peroxide.

Anatomic Diagnoses. Paraganglioma, benign, retroperitoneal.

Case 2

The patient was a Negro male, 34 years old, who was admitted to Detroit Receiving Hospital because of headaches of several months' duration associated with dizzy spells, nausea, and vomiting. He stated that he had innumerable tumors over his entire body since childhood. His blood pressure was 180/140 mm. Hg. The remainder of the physical examination was negative except for the cutaneous nodules and hypertensive retinopathy. Routine laboratory studies revealed little of note. A calcific mass, apparently within the left kidney, and a similar mass in the right upper abdominal quadrant were demonstrated radiographically. The patient was discharged and re-admitted 7 months later. It was then that the diagnosis of paraganglioma was suspected, for it was learned that laughing or change in his position would precipitate an attack of the previously described symptoms. Confirmatory tests were done, including perirenal insufflation for roentgenographic study. His attacks continued and he died, apparently in shock, before surgical treatment for his disease could be performed.

Multiple, discrete, cutaneous tumors varying from 1 mm. to 4 cm. in average diameter were distributed universally. There was an equinovarus deformity of the left foot. Examination of the contents of the cranial cavity revealed mild fibrosis of the leptomeninges. In the right cerebral hemisphere there was an elongated softening of the white matter. There was also a small hemorrhage in the pineal body. The heart weighed 425 gm. with a ventricular ratio of 3.0 (normal, 1.6 to 2.0). The peritoneal cavity contained 500 cc. of clotted and unclotted, recently shed blood. Beneath the right leaf of the diaphragm there was a hematoma, partially organized, measuring 8 cm. in maximum dimension. Within the spleen there was a subcapsular hematoma 4.5 cm. in diameter. In the walls of the esophagus, stomach, duodenum, small intestine, and large intestine were numerous firm, yellow-white tumors varying in size from 2 mm. to 2 cm. In the medial pole of the right suprarenal gland there was an encapsulated, spherical tumor, 3.5 cm. in diameter, which was reddish brown and hemorrhagic, and weighed 19 gm.; the uninvolved portion of the suprarenal gland weighed 5 gm. The opposite suprarenal body was diffusely enlarged, measuring 7 by 3 by 1 cm. and weighing 16 gm. Serial sections revealed no nodular lesion. Between this suprarenal body and the

superior pole of the kidney there was an area of recent hemorrhage measuring 4 cm. in maximum dimension.

In the brain there were atherosclerosis, arteriolar sclerosis, and thrombosis associated with multiple areas of softening and glial proliferation. The subcutaneous tumors and those throughout the gastro-intestinal tract were composed of encapsulated masses of interlacing bundles of fibrillar connective tissue with elongated nuclei, and no definite nerve bundles were seen in the sections examined. The lungs showed diffuse pulmonary edema, purulent bronchitis, passive congestion, and confluent lobular pneumonia. Nothing remarkable was noted in the sections prepared from the gastro-intestinal tract save for the previously described neurofibromas. In the kidneys, definite arteriolar sclerosis was seen which was indistinguishable from that frequently associated with essential hypertension. In addition a recent intracapsular hemorrhage was noted.

The suprarenal tumor was composed of polygonal cells measuring about 35 μ in maximum dimension which contained large vesicular nuclei with one prominent nucleolus per cell. The cytoplasm contained very fine, densely packed granules which were stained purple with hematoxylin and very closely resembled those of the secretory cells of the normal suprarenal medulla. These cells were arranged in cords and nests which were surrounded by a delicate connective tissue stroma. The tumor was highly vascular, containing many thin-walled capillaries and sinusoidal spaces, often markedly dilated. Some of the tumor cells were smaller (15 μ), more pink, and had dense nuclei without nucleoli. A portion of the tumor was placed in Orth's solution in order to demonstrate possible chromaffinity of the cells. When stained with Schmorl's technic [18] the nuclei appeared deep blue-green while the cytoplasm of the tumor cells contained numerous, very fine, yellowish green granules. Confirmation of this positive chromaffin reaction was obtained by staining formalin-fixed tissue with Heidenhain's azocarmine according to Gomori's suggestion.[17] With this technic, the granules were violet, corresponding to the granules of the suprarenal medulla which was used as a control. An interesting incidental feature was that the nucleoli of the tumor cells stained brilliant red.

Anatomic Diagnoses. Paraganglioma, benign, right suprarenal gland; neurofibromatosis, skin and gastro-intestinal tract; heart disease, hypertensive, with left ventricular hypertrophy; pneumonia, lobular, bilateral; hemoperitoneum; hematoma, subcapsular, spleen, kidney; arteriolar sclerosis, generalized; encephalomalacia; talipes equinovarus, left; kyphoscoliosis, thoracolumbar spine.

Case 3

The patient was a Negro male, 33 years of age, who was admitted to the Medical Service of the Detroit Receiving Hospital with a chief complaint of constipation, gas on the stomach, nausea, vomiting, and hiccoughs of 3 days' duration. He had had epigastric pain, relieved by soda and milk, for 10 years. The vomitus had been bloody on two occasions during the week prior to admission. He had had malaria as a boy and there was an indefinite history of syphilitic chancre 11 years before admission. Physical examination revealed slight icterus, decreased expansion of the right chest, and flatness. There was no cardiac hypertrophy; the abdomen displayed only slight tenderness, some distention, and spasticity of the rectus muscle on the right side. No abdominal masses were palpated. Results of routine examination of the blood and urine were normal. The working diagnosis was cholecystitis with cholelithiasis, and the patient was treated conservatively. During hospitalization, he became progressively worse and expired 5 days after admission.

The principal positive findings at necropsy were in the abdominal cavity. Generalized peritonitis had resulted from perforation of two ulcers of the duodenum and stomach. In the left suprarenal gland there was a neoplastic nodule measuring 2.5 cm. in diameter, confined to the gland. The remaining viscera appeared normal.

The neoplasm was composed of cells which varied greatly in size from 10 to 30 μ and were arranged in poorly formed cords and nests. The cells were roughly elliptical in most areas, but spherical in others. The cell boundaries were indistinct. The nuclei of the tumor cells, for the most part, contained moderately dense, diffusely distributed chromatin. However, the nuclei varied greatly in size and shape and contained no nucleoli. Some of the cells with indistinct outlines appeared to be giant cells and contained from two to five nuclei. The cytoplasm of the tumor cells was abundant and contained many fine, densely packed, violet-staining granules. Some of the tumor cells had undergone hydropic change. The stroma of the tumor, for the most part, was delicate, fibrous, and sparse. However, in some areas there was a very dense fibrous stroma. There were many dilated, thin-walled capillaries throughout the tumor. There was a moderate amount of necrosis and hemorrhage. At one edge of the section a rim of compressed suprarenal cortical tissue was seen.

Anatomic Diagnoses. Ulcers, duodenal and gastric, with perforation, generalized peritonitis, and abscess formation; paraganglioma, benign, left suprarenal gland.

Case 4

A white female, 21 years old, was first admitted to the Detroit Receiving Hospital on December 6, 1937. She had known that she had high blood pressure for 6 years prior to admission. This hypertension had been discovered in 1931 as part of a general physical examination because of symptoms of nervousness, palpitation, and headache. During the succeeding years the symptoms became progressively worse

and were accompanied by nervousness and irritability. In 1934 a partial thyroidectomy was performed at another hospital, at which time there was moderate elevation of the basal metabolic rate. Following this operation her symptoms were somewhat relieved but later returned, and in 1937 she was hospitalized for a "nervous breakdown." At this time her basal metabolic rate was plus 72 and another thyroid operation was performed. She felt better for a short time, but her symptoms returned and she became progressively worse. At the time of admission to the Detroit Receiving Hospital she was complaining of palpitation, headache, flushed face, and nervousness. Physical examination revealed only minimal changes of the vessels in the optic fundus. A remnant of the thyroid gland was palpable. The heart was moderately enlarged to the left with a systolic blowing murmur over the precordium and an accentuated aortic second sound. Blood pressure at the time of admission was 194/132 mm. Hg. Repeated determinations of the blood pressure revealed that the systolic pressure varied from 150 to 240 and the diastolic from 100 to 150. Routine studies of the blood and urine were normal. The Kline test was negative. The basal metabolic rate was plus 16. Blood cholesterol was 192 mg. per 100 cc. of plasma. An electrocardiogram indicated sinus tachycardia with no definite evidence of myocardial involvement. An excretory urogram revealed a slight outward displacement of the right kidney, and in the position of the left suprarenal gland a definite area of calcification was evident. While on the ward her condition remained unchanged. Marked variations in blood pressure determinations were noted. On January 18, 1938, an exploratory operation of the left suprarenal area was performed and a retroperitoneal tumor was found, situated superiorly and mesially to the upper pole of the left kidney and extending posteriorly along the spinal column at approximately the level of the 12th thoracic vertebra. The anterior surface was covered by a complicated mass of veins of considerable size, some being 2 mm. in diameter. Intravenous fluids, including whole blood, were administered. The blood pressure fluctuated greatly; at one time the systolic pressure was 290 and a reading was unobtainable at another time. All of the tumor was removed except the extreme posterior portion lateral to the spine. At this point in the operation the patient stopped breathing, and quantities of rust-colored, frothy fluid exuded from the nostrils and throat. Despite supportive measures, she expired. Permission for necropsy was not granted.

The specimen was submitted in several pieces, having an aggregate weight of 20 gm. They were irregular, dark brown, and soft. The formalin in which the tissue had been immersed for 24 hours was discolored brown. The tumor cells were large, irregular, and contained hyperchromatic nuclei with abundant deep-staining cytoplasm. Many of the cells were vacuolated. There was a considerable variation in nuclear size and staining intensity. A few mitotic figures were seen. Some of the cells were multinucleated, containing two to four nuclei. Thick and thin-walled veins were present throughout the tumor. There was an incomplete, thick, fibrous capsule. A portion of the tumor was stained with Giemsa's stain and no olive-green granules were seen. This, then, would be a negative chromaffin reaction with the Schmorl method.[18] However, when the tissue was stained with azocarmine, there was a definite positive reaction, the cytoplasm of the tumor cells

containing gray-blue, coarse granules. The nuclei were red except the large vesicular ones which had prominent red nucleoli.

Anatomic Diagnosis. Paraganglioma, benign, retroperitoneal.

Case 5 *

The patient was a white woman, 50 years of age, who had suffered from dyspepsia for many years. During the past 2 years she developed a chronic, persistent hypertension with blood pressure of approximately 160/90 mm. Hg. There was a gradual onset of exertional dyspnea and ankle edema which were relieved by the administration of digitalis. Four days prior to hospitalization she was seized by an attack of nausea, vomiting, and severe pain in the right upper quadrant. At the time of admission to Woman's Hospital her temperature was 100.2°F.; pulse rate, 90 per minute; and blood pressure, 190/110 mm. Hg. There was tenderness on the right side of the abdomen associated with a poorly defined mass. The results of hematologic and urine studies were within normal limits. On November 21, 1936, 6 days after admission, a cholecystectomy was done. During the operation, the blood pressure fluctuated widely. The gallbladder was thickened and contained two large stones and a large amount of thick purulent material. Her postoperative course was satisfactory during the first 3 days. She then became apprehensive, nauseated, and vomited several times. Temperature, pulse rate, and respirations increased and she became comatose. She died 9 hours after the onset of these symptoms.

There was a fairly well developed growth of hair on the upper lip and chin, but no other signs of masculinization. The heart weighed 300 gm., but was otherwise normal. There was hemorrhagic edema of the lungs. The right suprarenal gland was diffusely enlarged and weighed 25 gm. The left suprarenal gland was replaced by a firm, encapsulated tumor which weighed 600 gm. and measured 11 by 10 by 8 cm. It was easily removed from the surrounding structures. On cut section, it was seen to be highly vascular, soft, and white in some areas and pink in others. The epinephrine content was ascertained according to the method of Folin, Cannon, and Denis [19] and it was found that 10.2 gm. of the tumor contained 24.6 mg. of epinephrine. This would indicate that the entire tumor contained about 1400 mg. of epinephrine.

In the sections prepared from paraffin-embedded tissue and stained with hematoxylin and eosin, the tumor cells were seen to be polyhedral with indistinct cell boundaries. The average size of the cell was 35 μ. The nuclei in most cases were large, vesicular, and nearly all of them had prominent nucleoli. Some cells appeared to be binucleated. The cytoplasm contained fine, violet-staining granules. The cells were arranged in cords and nests and were separated from one another by delicate connective tissue stroma with a rich capillary network. Some

* Reported with the permission of Drs. Earl G. Krug and D. C. Beaver.

of the capillaries were distended and contained very many white blood cells. There was a remarkable resemblance between the microscopic pattern of the tumor and the normal suprarenal medulla. There was no necrosis or hemorrhage. The capsule was incomplete. Many thin- and thick-walled veins were present. A portion of the tumor was fixed in a chromate solution and sections examined from this material revealed brown granules in the cytoplasm of the neoplastic cells.

Anatomic Diagnoses. Paraganglioma, benign, left suprarenal gland; hypertrophy, myocardial, mild; edema, hemorrhagic, lungs; gallbladder, absence of, acquired.

Review of the Literature

The three terms commonly used in the literature by which these tumors are designated are paraganglioma, pheochromocytoma, and chromaffinoma. For reasons previously stated, chromaffinoma seems objectionable. Pick,[20] in 1912, suggested that the term pheochromocytoma (tumor of dark-colored cells) be used for those tumors arising in the suprarenal medulla and that the extra-suprarenal tumors be called paragangliomas. Some authors have followed his suggestion, but since this separation is arbitrary and does not reflect a fundamental distinction, we prefer to designate all of these tumors as paragangliomas. This term seems most acceptable since it refers to the cells from which these tumors are derived.

Many excellent reviews of the literature have appeared during the past 15 years. Belt and Powell [21] collected all of the cases reported up to 1934. In 1941 Biskind, Meyer, and Beadner [22] contributed a compilation of the cases treated surgically which had been reported prior to that time. Rosenthal and Willis [23] reviewed all of the cases of paraganglioma associated with neurofibromatosis. Green [24] studied the incidence of chronic hypertension and analyzed 50 reported cases. McGavack, Benjamin, Speer, and Klotz [25] analyzed the reports of malignant paraganglioma. Mackeith,[26] in his article submitted for publication in May, 1943, stated that 165 of these tumors had been described. Since that time approximately 40 more cases have been added. The 5 cases described in this paper bring the total number of reported cases to about 210.

These tumors occur with equal frequency in the male and the female, chiefly during young adult life, and usually between the ages of 20 and 50 years. However, the age variability is great: one of Fingerland's [27] cases was that of a 71-year-old male, and Soffer, Mencher, and Colp [28] reported an active tumor occurring in a 7-year-old girl. The few reports of cases occurring in infants and very young children are

all highly atypical. The 1-year-old infant described by Neff, Tice, Walker, and Okerblad [29] displayed hirsutism, precocious development of the genital organs, and obesity. The tumor which occurred in the mediastinum of a 4-year-old boy, reported by Wahl and Robinson,[30] contained neuroblastic elements with widespread metastases and thus does not belong in the category of paragangliomas.

About 90 per cent of these neoplasms have occurred in the suprarenal glands and there is a slight, but definite, predilection for the right gland. All of the extra-suprarenal tumors have occurred in the abdominal cavity or retroperitoneal area except two. One of the two acceptable intrathoracic paragangliomas was reported by Philips [31] from the apex of the pleural cavity, apparently arising from the first left thoracic ganglion. The second was reported by Miller [32] as occurring in a paravertebral position in the right pleural cavity opposite the 6th interspace. Of those described as extra-suprarenal but intra-abdominal, 10 have occurred in the organ of Zuckerkandl, 3 in the retroperitoneal area (including our first case), one at the hilus of a kidney, and one at the celiac ganglion. Many others have been reported in the carotid body, the appendix, and the sacrococcygeal body, but these tumors are not included in this review. Waaler [33] has collected all of the extra-suprarenal paragangliomas reported up to 1945.

These tumors are multiple in about 10 per cent of the cases. In 18 reported cases they have occurred simultaneously in both suprarenal glands. The importance of this fact is emphasized in the report of Knake [34] in which is described an 11-year-old boy who had one suprarenal tumor removed but died 5 weeks later. A similar benign tumor was found in the opposite suprarenal gland at necropsy. In addition to those with bilateral tumors, other reports describe apparently benign but multiple tumors. One of Fingerland's [27] cases had a tumor of the right suprarenal gland and the organ of Zuckerkandl. The important clinical implications of these facts must be apparent.

The tumors have varied in weight from 5 gm. to more than 2 kg. One tumor described by Soffer *et al.*[28] weighed 2 kg. after much blood had been removed. Typically, the tumors are encapsulated, spherical or oval, and reddish brown. Many are hemorrhagic and necrotic, and have undergone cystic degeneration. Often a rim of suprarenal cortex can be seen at one edge of the specimen. In our case 1 the tumor was blue-black because of the high melanin content, but this is unusual.

In the tumors which develop the more typical and characteristic histologic pattern there is an unmistakable resemblance to the suprarenal medulla. The nuclei of the tumor cells are large, vesicular, and frequently have prominent nucleoli. The cytoplasm is abundant, mak-

ing each cell polygonal. Often fine granules can be seen in the cytoplasm, even in sections prepared from formalin-fixed tissues and stained with hematoxylin and eosin. Frequently the cells are arranged in cords and nests and are separated by a delicate connective tissue stroma. Usually, there is an abundant capillary network. When fixed in Orth's solution and stained by the Schmorl technic [18] the nuclei are blue and the cytoplasm often contains olive-green granules. When fixed in formalin and stained with azocarmine, as suggested by Gomori,[17] the nucleus usually is pale, the nucleolus a brilliant red, and the cytoplasm often contains bluish violet granules. However, many of the tumors are atypical and may pose a problem in histologic diagnosis. Some tumors are composed of small cells with dark, pyknotic nuclei and no demonstrable nucleoli. The cells may be fusiform or spindle-shaped. Great variation in size and shape may be present, including the formation of giant cells. The cytoplasm may be scanty and contain no granules. The reactions to the Schmorl [18] and azocarmine stains may be negative. The cells may contain a large amount of melanin. Ganglion cells may be present. The pathologist must then give special consideration to the clinical features of the case.

It is difficult to ascertain the real incidence of hormonal activity of these tumors. Before 1922, at which time the relationship of these tumors to paroxysmal hypertension was first clearly described,[4] most of the reports consisted of necropsy findings and about one-half of the tumors apparently were inactive. During the past 20 years many clinical as well as pathologic diagnoses have been made and, quite naturally, the percentage of hormone-producing tumors has increased. During the past 4 years about 30 cases have been reported, all characterized by symptoms of epinephrine production. It is interesting that of the 9 tumors associated with generalized neurofibromas, only 2 (including our case 2) have produced the suprarenal-sympathetic syndrome.

There is some confusion in the literature concerning the frequency with which these tumors are malignant. McGavack *et al.*[25] stated that only 8 cases of definitely malignant paraganglioma had been reported and that all of these were physiologically inactive, *i.e.*, did not produce the suprarenal-sympathetic syndrome. The following year Mackeith [26] stated that 17 malignant paragangliomas had been described and that some of them had been active. The reason for this disagreement is that Mackeith apparently accepted all of the cases reported as malignant, whereas McGavack critically analyzed all of the reported cases and accepted only about half of them. McGavack categorically stated that it is not possible to separate the malignant from the benign

tumors histologically, and that "all of the cases in which metastases did not occur should be classified as benign." On this basis, he accepted only 7 cases and added another. We would insist upon invasion in lieu of metastasis as a criterion of malignancy. We believe that Fein and Carman's [35] case is a typical example of a benign paraganglioma incorrectly diagnosed. This patient was a 27-year-old female; at necropsy an encapsulated tumor of the right suprarenal gland was found which was assayed and found to contain epinephrine. There was no cachexia and no local invasion by the tumor or metastasis. Furthermore, the photomicrographs revealed a pattern which is compatible with that of a benign paraganglioma although the diagnosis given is medullary carcinoma of the suprarenal gland (malignant pheochromocytoma). Many similar examples could be cited to account for the disparity between the figures of Mackeith and McGavack. Since McGavack's report, no acceptable malignant paraganglioma has been reported. The presence of multiple tumors does not constitute proof of malignancy. There have been 18 reported cases of bilateral paragangliomas. McGavack has accepted as malignant only 5 cases with bilateral tumors. The bilateral paragangliomas of the suprarenal gland reported by Knake [34] were clearly benign. Fingerland's [27] case in which tumors were found in the right suprarenal gland and the organ of Zuckerkandl was reported as one of benign neoplasm.

In summary, it can be stated that a paraganglioma which is locally invasive or has metastasized should be considered malignant. If there are multiple tumors and one or more of the nodules is located where paraganglion cells are never found normally (*e.g.*, lymph node or bone), it is a malignant neoplasm. If the tumor is well encapsulated but the cells appear to be anaplastic, the observer must be cautious in classifying it as malignant. If the tumors are multiple and all of them are situated where paraganglion cells are normally found (*e.g.*, both suprarenal glands, one suprarenal gland and the organ of Zuckerkandl), again one must be cautious in interpreting one tumor as being primary and the others metastatic.

For the 8 cases of malignant paraganglioma accepted by McGavack *et al.*,[25] the following features were observed: All occurred in the suprarenal gland; none was associated with paroxysmal hypertension or the suprarenal-sympathetic syndrome; the age range was from 30 to 68 years with an average of 45.5 years; 5 of the 8 were bilateral; the outstanding clinical features were loss of weight, cachexia, and pain at the primary and metastatic tumor sites. In McGavack's own case, the picture of cachexia was so extreme that diagnoses of Addison's and Simmond's diseases were considered. In 3 of

the 8, a palpable mass was present. The regional lymph nodes were involved in all. Other metastatic sites were as follows: Thoracic lymph nodes, 5; liver, 4; bones, 3; lungs, 3; pleura, 2; skin, 2; intestines, 1; kidney, 1.

In several of the reported cases increased pigmentation of the skin has been described. There are two possible interpretations of this observation. The more obvious is that the expanding tumor has produced pressure on the suprarenal cortex and that the increased pigmentation is a result of cortical deficiency. There is abundant evidence, both clinical and experimental, that deficiency of suprarenal cortical hormones causes deposition of melanin in the skin. At least 4 cases of paraganglioma have been reported in which clinical features of Addison's disease were present.[25] In 2 there was excessive loss of sodium chloride in the urine and a retention of potassium. But the disturbing fact is that in none of these cases could encroachment on the suprarenal cortex be demonstrated. In McGavack's [25] case, for example, there was stretching of the cortex on the side of the tumor, but the opposite suprarenal gland was uninvolved, and serial sections of all endocrine glands revealed no significant change. The cause for the development of Addison's syndrome remains obscure. The other possible interpretation of the increased skin pigmentation is that it is related to neurofibromatosis, it being well known that areas of deep pigmentation often precede the development of neurofibromas.

Before concluding a discussion of the relationship of paragangliomas to disturbed suprarenal cortical metabolism, mention should be made of LeCompte's [36] case of a 31-year-old white female who displayed the signs and symptoms of the androgenital syndrome. Since this syndrome is sometimes due to hyperfunction of the suprarenal cortex, it might be thought that stretching of the suprarenal cortex stimulated it to activity, an effect exactly the opposite of that suggested in the preceding paragraph to explain the production of Addison's syndrome.

Many causes and mechanisms of death have been described for patients with paragangliomas. Of those with malignant tumors the cause of death was attributed to the cancer. Patients with tumors which were nonfunctional and apparently produced no epinephrine died of unrelated causes. When the epinephrine-sympathetic syndrome was present, the cause of death was usually attributed to some effect which the tumor produced. Very often the anatomic changes at death are minimal and the possibility of acute epinephrine intoxication arises. Dolgin [37] reviewed the causes of death in some detail and cited the work of Raab [38] on the toxic effects of epinephrine-like substances.

Effects of Epinephrine

One of the most interesting aspects of these tumors, and certainly the one most widely discussed in the literature, is the physiologic change produced in the patient as the result of the production of an epinephrine-like substance. In this respect paragangliomas resemble the active islet cell tumors of the pancreas, the virilizing tumors of the ovaries and suprarenal cortex, the basophilic tumors of the pituitary body, and others. The first clear-cut description of the syndrome of paroxysmal hypertension was written in 1922 by Labbé, Tinel, and Doumer.[4] Following this report there was rapid advancement in knowledge of the activity of these tumors. For several reasons it was natural for the early workers to suspect that they produced a substance similar to epinephrine. The frequent occurrence of these tumors in the suprarenal gland, the histologic similarity to the suprarenal medulla, the chromaffin reaction, and the striking resemblance of the spontaneous attacks of headache, hypertension, sweating, and pallor to the known physiologic effects of epinephrine all pointed to production by the tumor cells of a substance similar to epinephrine. That a vasopressor substance is present in an active paraganglioma can be demonstrated by several methods. The one most commonly used has been the injection of a neutralized acid extract of the tumor into a lightly anesthetized dog and a comparison of the pressor effect produced with the action of a known quantity of epinephrine. Hyman and Mencher [39] prepared a solution from a patient's blood while she was having an attack of paroxysmal hypertension. By perfusion of this material through a rabbit's ear, they were able to demonstrate a pressor effect. That this substance was similar to epinephrine was further supported since the effect could be reversed by ergotamine. In several cases an elevated level of the blood potassium has been reported. The significance of this is apparent when one considers that the injection of epinephrine into a human being causes an elevation of blood potassium of as much as 87 per cent above normal.[26] Epinephrine in a crystalline form was obtained from a tumor by Kelly, Piper, Wilder, and Walters [40] in 1936. DeVries, Mandl, Rachmilevitz, and Ungar [41] used a colorimetric method (Ewins' [42] test in a buffered solution) in estimating the epinephrine content of the tumor to be studied. Mortell and Whittle [43] injected dogs intravenously with diluted fluid obtained from a cystic tumor which they encountered, and made direct measurements of the vasopressor effect. Furthermore, they cited Strömbeck and Hedberg's [44] description of a chemical method based on the decolorization of methylene blue. The latter had described a case of which, during the

paroxysms of hypertension, the blood contained one thousand times the normal amount of epinephrine and during the remissions showed an epinephrine content which was elevated thirty-fold. It is true that these methods are often indirect and leave much to be desired, but certainly they prove that epinephrine (or a substance closely allied to it) is often produced by these tumors.

It is usually stated that bio-assay of the tumor must be done shortly after it has been removed. However, nowhere have we found descriptions of decreasing vasopressor activity as the time interval between removal and determination of epinephrine increased. In Mortell and Whittle's case [43] there was no decreased potency at the end of 48 hours, when the tumor was stored in the ice box. Fingerland [27] reported 2 cases in which he tested the formalin, alcohol, and Bouin's solution in tumors which had been preserved for 6 years, and demonstrated a strong epinephrine reaction. Since epinephrine is readily destroyed by oxidation in alkaline solution, one should store material to be assayed in an acid solution. The amount of pressor substance contained in paragangliomas has been reported to vary from 6.7 to 2300 mg.[45] From the normal human adult suprarenal glands only about 8 mg. are recoverable.

Varied clinical effects may be produced by these tumors. The syndrome first described and the one most often seen and recognized is that of paroxysmal hypertension. More recently, excellent reports have been made of cases simulating chronic essential hypertension,[24, 46] diabetes mellitus,[47] hyperthyroidism,[45, 48] and adynamic ileus.[49] Some patients have exhibited signs and symptoms of several of these categories. Rodin [50] has described in great detail the ophthalmologic changes which occur with this disease. Mackeith [26] has discussed the electrocardiographic changes. In Mortell and Whittle's [43] case, electroencephalograms were taken during the paroxysms and were found not to be altered. Hyman and Mencher [39] measured temperatures of the peripheral skin and found them to be lowered, presumably because of vasoconstriction. In the 2 cases reported by McCullagh and Engel [48] an elevated urea clearance was demonstrated, which suggests increased renal blood flow; in one there was also severe polyuria, arousing a suspicion of diabetes insipidus.

Several tests have been devised which have been useful for studying a patient suspected of having a paraganglioma. All of them are similar in that they are attempts to provoke an attack. One of the first was suggested by Coller, Field, and Durant [51] and consisted of a subcutaneous injection of a minute amount of epinephrine. Roth and Kvale [52] have induced attacks with the use of histamine. The mechanism is thought to depend upon the fact that histamine is antagonistic

to epinephrine and causes a temporary reduction in the circulating amount of that substance. However, the effect is temporary and thus the tumor responds with a "rebound" liberation of a large amount of epinephrine. Among the other methods might be mentioned the immersion of the feet in cold water (cold pressor test), the injection of insulin, and hyperventilation (which is thought to cause the diaphragm to press on the tumor). All of these tests have been successful in some cases and unsuccessful in others, so that there is little to recommend one rather than another.

Perirenal air insufflation preparatory to radiographic examination has been advocated by Hyman and Mencher [39] and has been very useful in their hands. Others have hesitated to employ this procedure because patients with these tumors are unstable and disastrous results have ensued. It may be that the attempted perirenal insufflation for the patient of our case 2 had something to do with his demise. Scout films of the abdomen have been helpful in many cases, for calcification in the kidney region has been seen. Often these shadows have been misinterpreted as being tuberculous in origin.

Relation to Neurofibromas

In the second case of our series, an active paraganglioma of the right adrenal was associated with neurofibromatosis of skin and viscera. Suzuki [3] was the first to report the simultaneous presence of these two neoplastic diseases, and the literature now contains 8 previously reported cases. In only one of these was the paraganglioma active, producing the suprarenal-sympathetic syndrome.[53] Our case 2, then, is the second to be reported in which a paraganglioma, multiple neurofibromas, and paroxysmal hypertension have occurred together.

The co-existence of these two neoplastic conditions in a frequency of 1 to 23 suggests that the relationship is more than fortuitous. Nearly all authors reporting such cases have made this comment, but nowhere have we found a satisfactory explanation of this concurrence. As has been described above, the paraganglion cells are derived from the migrating undifferentiated cells of the neural crest. Harrison [54] has demonstrated experimentally that when the neural crest is divided from the neural tube, the motor nerves continue to develop, but are devoid of the normal sheaths (Schwann cells). This indicates that the sheath cells, like the paraganglion cells, are derived from the neural crest. Stout [55] recently reviewed the histogenesis of neurofibromas and stated that Schwann cells play the dominant rôle in the formation of these tumors. These accumulated facts suggest, then, that paragangliomas and neurofibromas are both derived from cells which have ultimately come from the neural crest. Several writers have reported

cases demonstrating tumors consisting of combinations of ganglioneuromas, neuroblastomas, and paragangliomas.[30, 56, 57] These have been accepted as being expressions of different degrees and directions of differentiation from common primordial cells. An extension of this concept might explain the concomitant occurrence of neurofibromas and paragangliomas.

Melanin Production

One of the cases reported by us (case 1) is a retroperitoneal paraganglioma which was deeply pigmented. In attempting to identify the pigment, four substances were considered: hemosiderin, lipochrome, chromaffin granules, and melanin. The first three were eliminated from consideration and the pigment interpreted as melanin because it was iron-free, not soluble in fat solvents, bleached with hydrogen peroxide, and darkened with 5 per cent aqueous silver nitrate. Baker [58] has shown that these are the only chemical reactions which are of much practical value in the identification of brown pigment.

Tyrosine, when oxidized in the presence of the enzyme tyrosinase, yields melanin.[59] It can also be used as the precursor of epinephrine.[60] The possibility of a similar source for melanin and epinephrine and the close chemical relationship of these substances make it seem reasonable that a neoplasm possessing the potential capacity to produce epinephrine might also produce melanin.

Millar [61] has reported a case of malignant ganglioneuroma of the 7th thoracic sympathetic ganglion in which the ganglion cells contained melanin. Masson [62] has traced the origin of melanomas of the skin to Meissner's corpuscles which are composed of modified Schwann cells. Hyperpigmentation of the skin occurs before the development of, and in association with, multiple neurofibromas. Thus it would appear that a tumor derived from the cells which have origin from the neuroectodermal cells of the neural crest (paraganglioma, ganglioneuroma, neurofibroma, and ordinary melanomas of the skin) is apt to produce melanin. We are well aware that the definitive histogenesis of all of these tumors is not finally settled, but we believe that the accumulating facts lend support to the opinion of a common origin for these diverse neoplasms.

Summary and Conclusions

On the basis of the origin and distribution of paraganglion cells, tumors arising in the carotid body, appendix, and coccygeal body have been excluded from the group of paragangliomas. The chromaffin reactions are not specific for paragangliomas.

The previously reported cases of paraganglioma, reviewed with regard to age and sex distribution, location of tumors, hormonal activity, and histologic appearance, exhibit a variability of cytologic pattern and a marked degree of pleomorphism, which is, nevertheless, consistent with a diagnosis of benign paraganglioma. A diagnosis of malignancy should not be made in the absence of invasion or metastases.

There are 9 cases in which paragangliomas have occurred concurrently with neurofibromatosis. This concomitance may depend upon different degrees and directions of differentiation of neural crest cells.

Of the 5 original cases of paragangliomas which are described, one tumor was retroperitoneal and contained a brown pigment which was identified as melanin. The close chemical relationship between melanin and epinephrine is probably significant in this connection.

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DESCRIPTION OF PLATES

Plate 192

Figs. 1 and 2. Case 1. External and cut surfaces of paraganglioma.

Fig. 3. Case 1. Pigmented area in tumor. Hematoxylin and eosin stain. × 240.

Fig. 4. Case 1. Photomicrograph of nonpigmented area in tumor. Hematoxylin and eosin stain. × 240.

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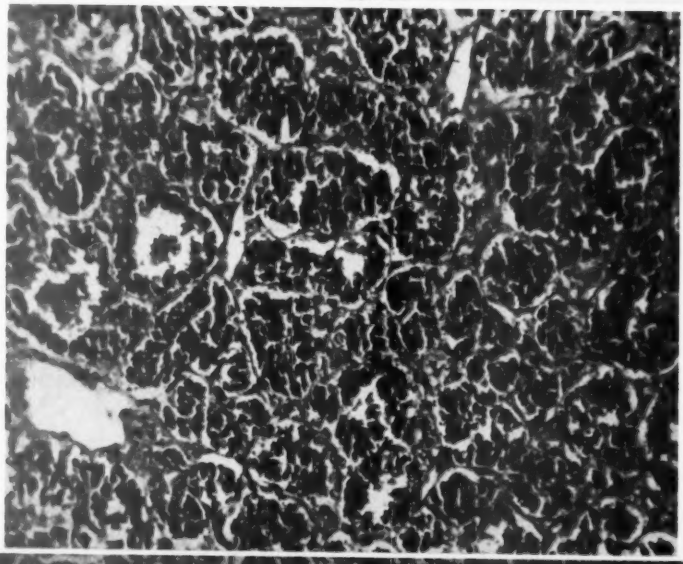

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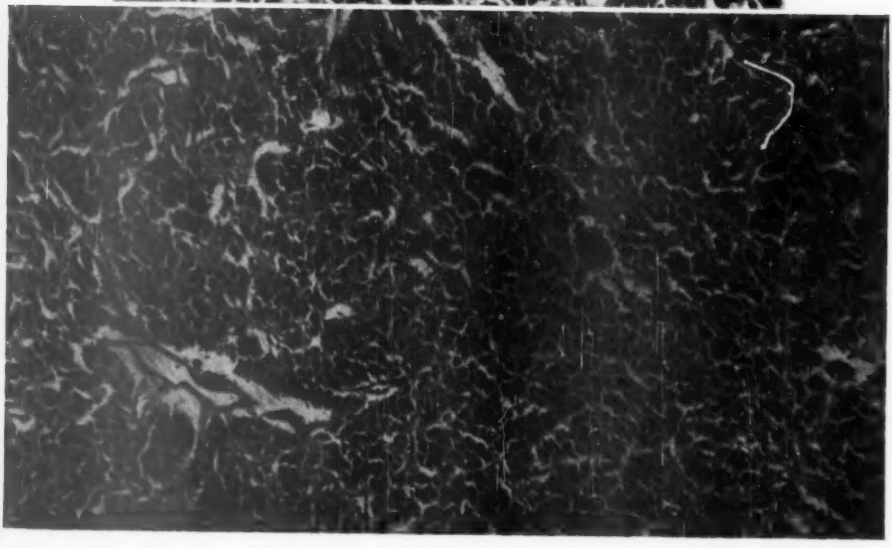
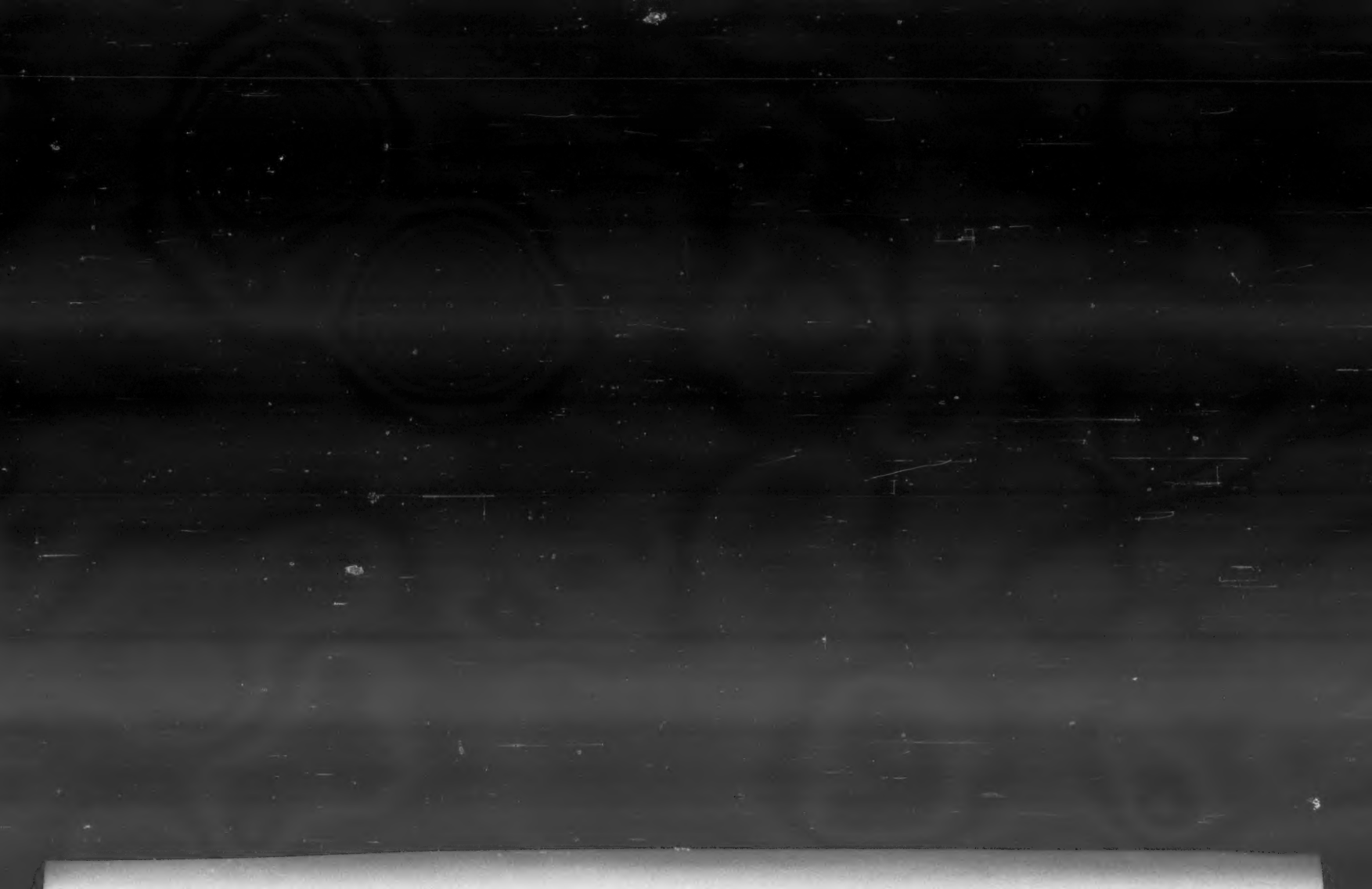


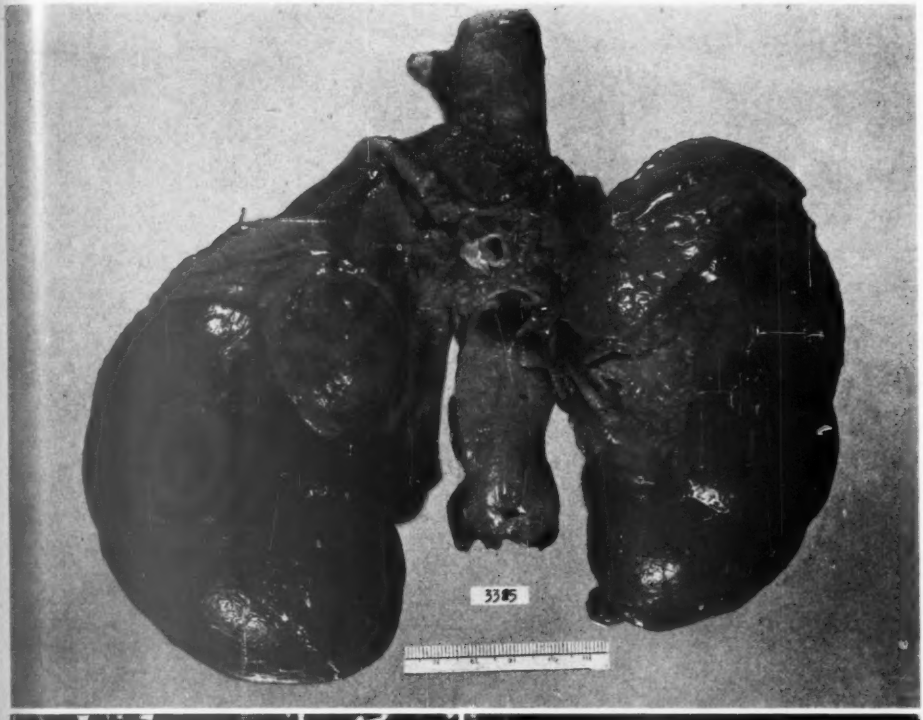
PLATE 193

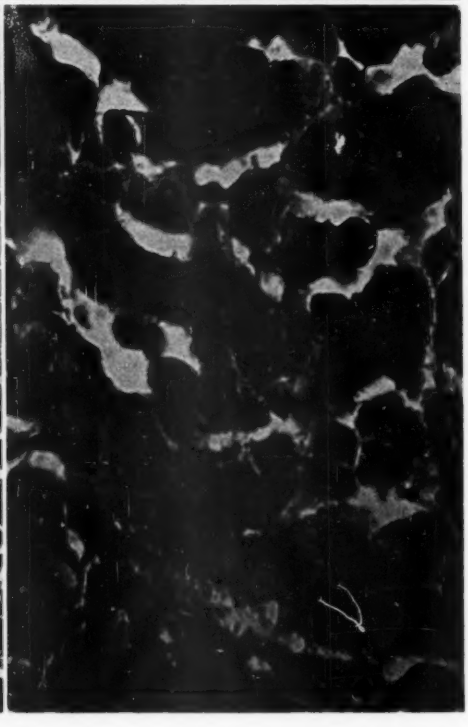
FIG. 5. Case 2. Paraganglioma of right adrenal; diffuse hypertrophy of opposite adrenal.

FIG. 6. Case 2. Photomicrograph of representative area of the neoplasm. Hematoxylin and eosin stain. × 240.

FIG. 7. Case 2. Cytoplasmic granules and prominent nucleoli in tumor cells. Azocarmine stain. × 516.







7

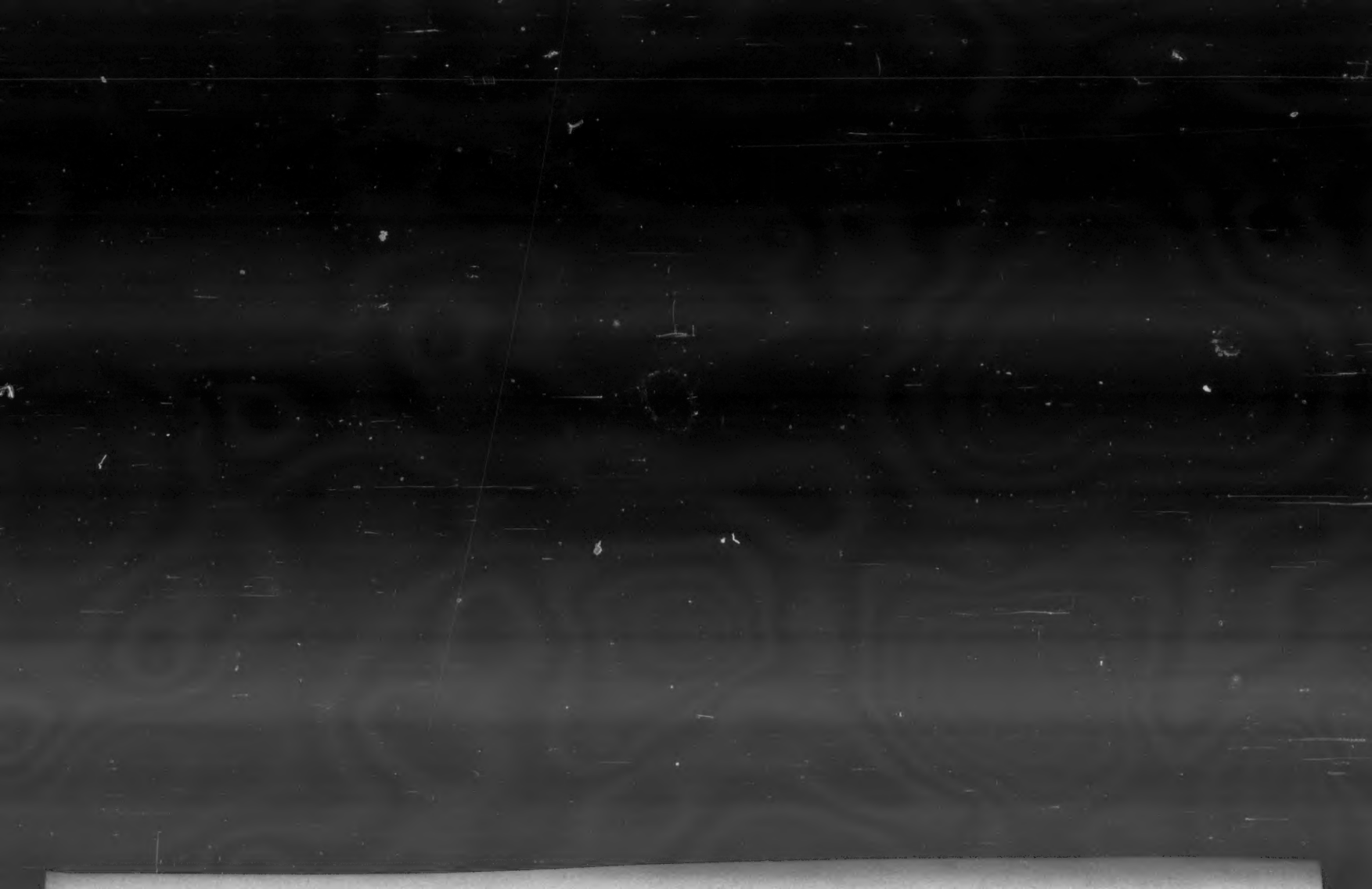
Brines and Jennings Paragangliomas

PLATE 194

FIG. 8. Case 3. Photomicrograph of neoplasm showing hydropic cell area at upper right and granular cell area at left. Hematoxylin and eosin stain. × 120.

FIG. 9. Case 3. Multilocular vacuolation of cytoplasm in hydropic area. Hematoxylin and eosin stain. × 516.

FIG. 10. Case 3. Granular cytoplasm in darker staining areas. Hematoxylin and eosin stain. × 516.



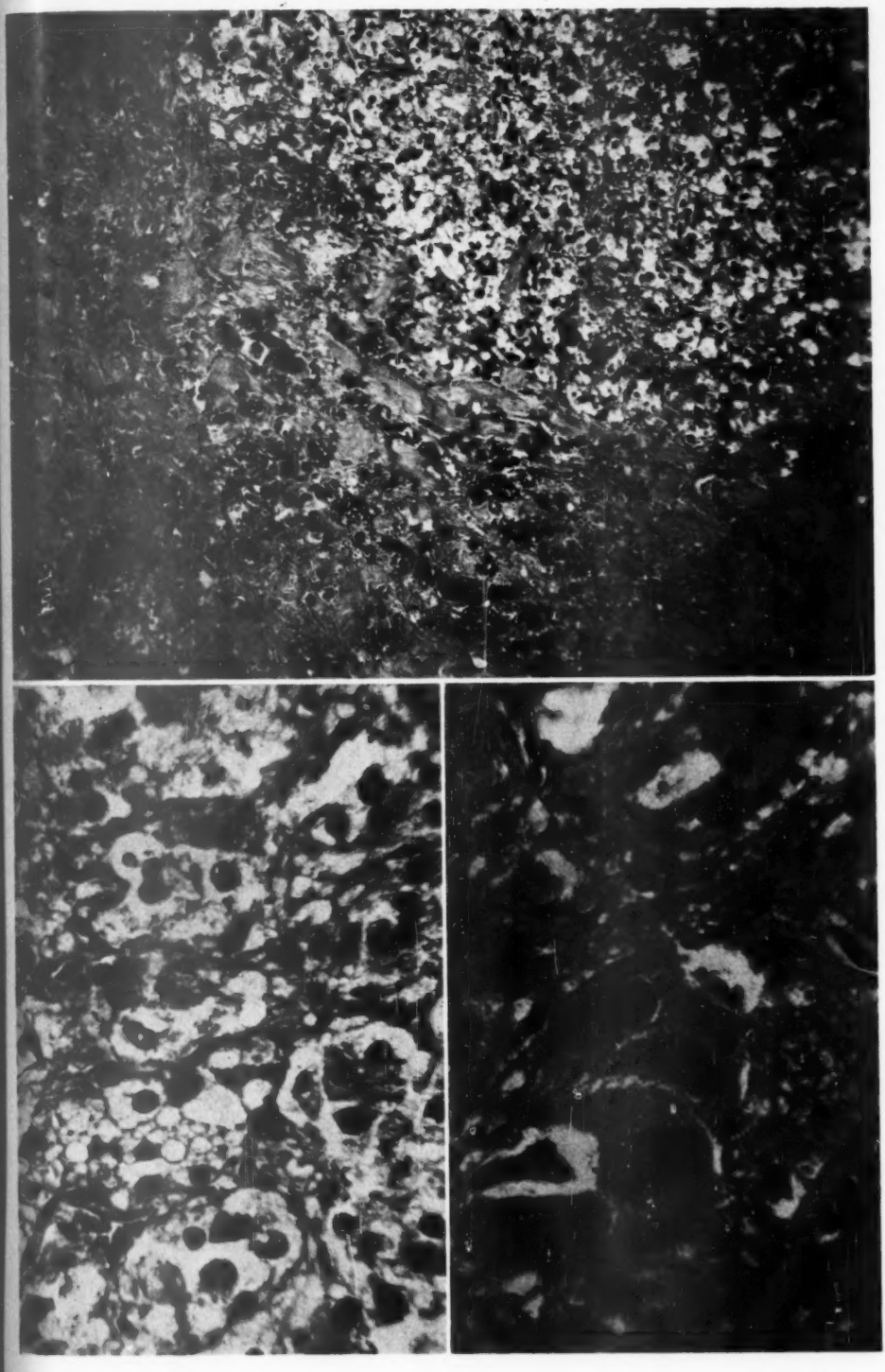


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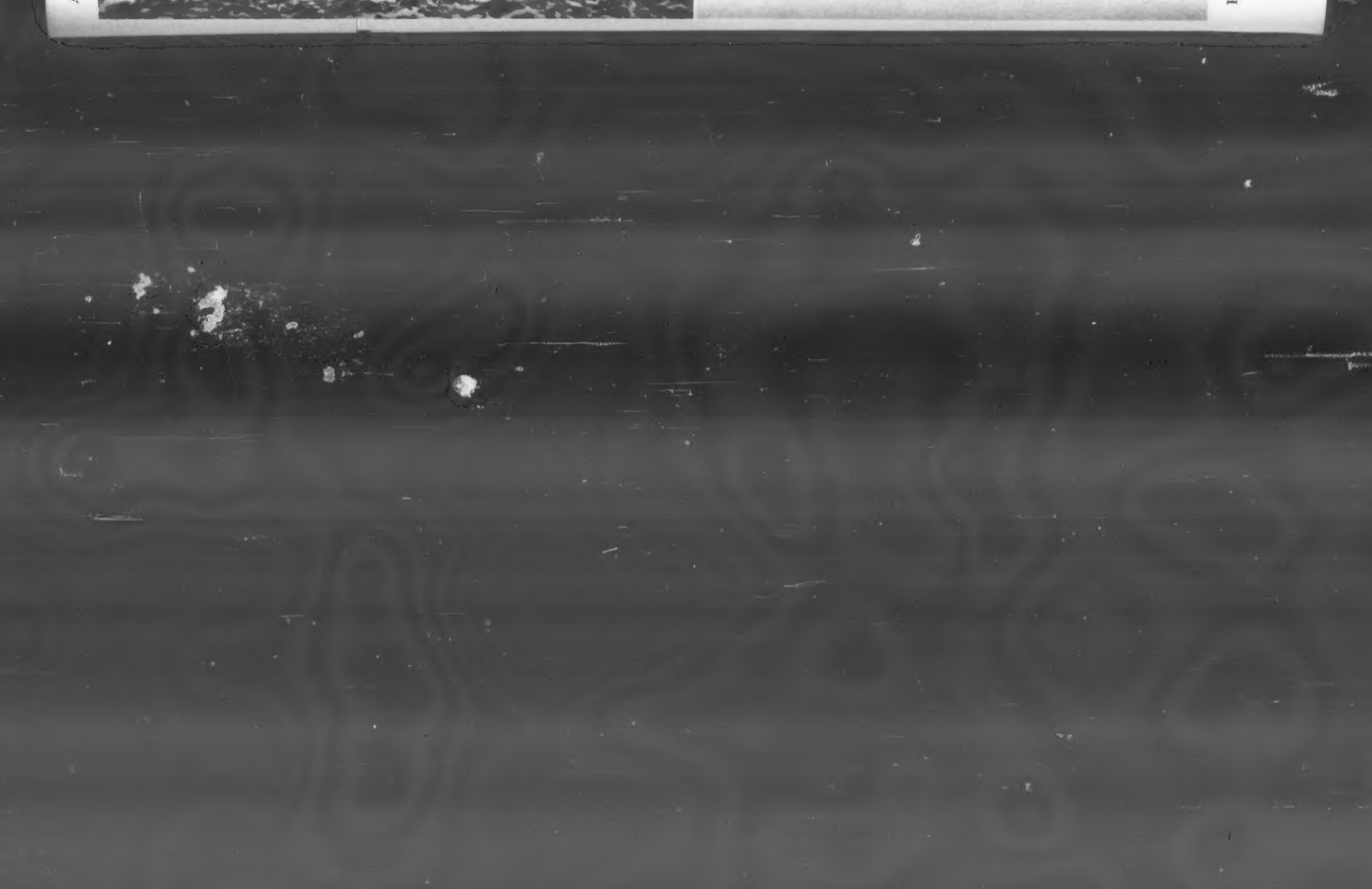
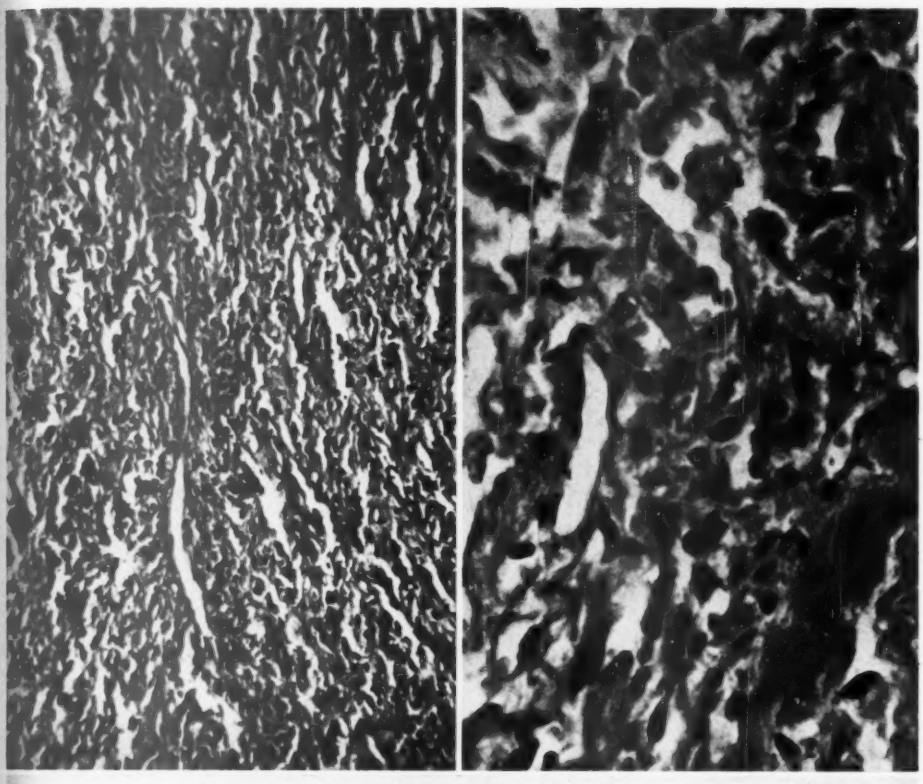
PLATE 195

FIGS. 11 and 12. Case 4. Photomicrographs of representative areas. Many oval and spindle-shaped cells; some pleomorphism and prominent vascular spaces. Hematoxylin and eosin stain. × 125 and × 512.

FIG. 13. Case 5. Photograph of paraganglioma of left adrenal gland.





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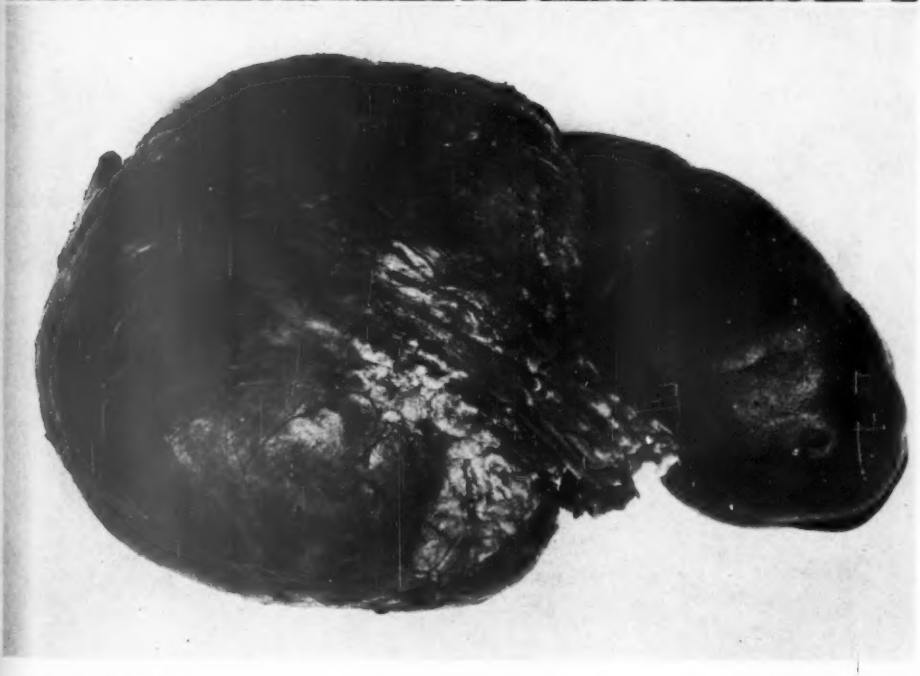

PLATE 196

FIG. 14. Case 5. Photomicrograph of representative area of the neoplasm. Hematoxylin and eosin stain. × 240.

FIG. 15. Case 5. Distinct granules in cytoplasm and wide sinus-like vascular spaces. Hematoxylin and eosin stain. × 512.

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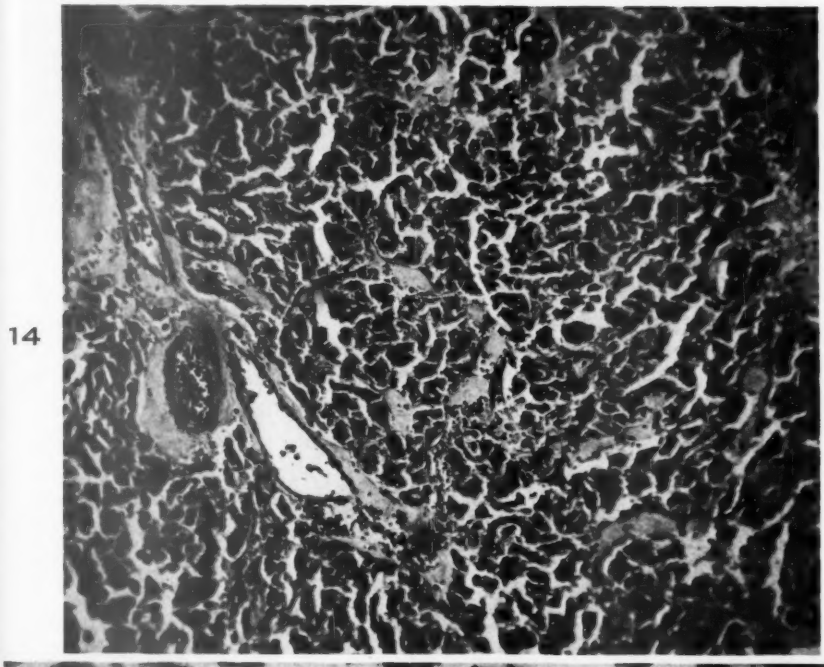

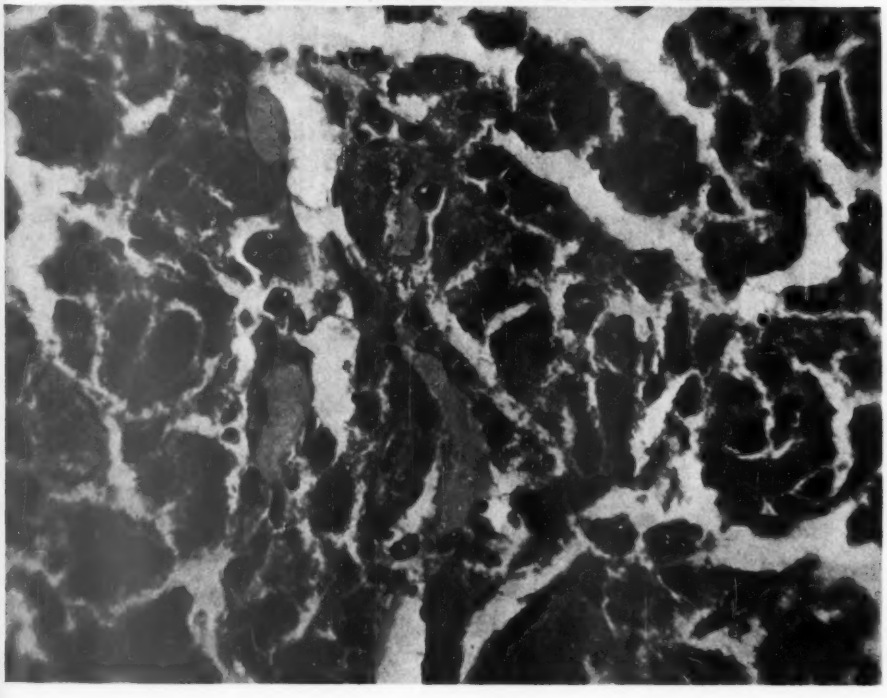

THE CYTOLOGIC FEATURES OF CARCINOMAS AS STUDIED BY DIRECT SMEARS *

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During the past 20 years the method of diagnosing cancer by visual examination of a single cell or a group of isolated cells has received more and more attention.[1-5] The method is based upon the assumption that morphologic as well as functional differences distinguish cancerous from noncancerous cells. The efficacy of the cytologic approach to the problem of tumor diagnosis is not yet proved. Its limitations and advantages are in part unknown, and only by controlled examination of large groups of tumors and normal tissues and experimentation with different methods will they be established. Therefore, this study was undertaken.

Almost since the advent of Virchow's cellular pathology attempts have been made to identify the characteristics of the cancer cell. The older work in this field was comprehensively reviewed by Quensel [4,6,7] (1928) with particular emphasis upon the cytologic features of malignant cells in exudates. In the recrudescence of interest in this method of diagnosis, MacCarty [2,8-10] with his group [11-14] in the United States and Dudgeon [1,15,16] with others [17-19] in England have been leaders in their respective countries. The development of the technic of needle biopsy of tumors [20-26] and the work of hematologists in the study of bone marrow,[27-31] lymph nodes,[32-39] and spleen [40] have all lent further impetus in this direction. Particular recognition must be extended to Papanicolaou [3,41,42] and others,[43-54] who in recent years have cultivated the method of single cell diagnosis to a point where it has found widespread practical application, particularly in examination of body excretions.

Despite this mass of evidence, the value of cytologic diagnosis of tumors is not widely acknowledged. The greatest skepticism is found among pathologists, and not without reason. Although there is a great difference between Borst's [55] statement, "Die Geschwulstzelle, auch die bösartige, hat weder in morphologischer, noch in chemischer, noch in irgend einer anderen Hinsicht etwas absolut Charakteristisches an sich," and that of MacCallum,[56] "No doubt, in time we shall have a reliable morphological criterion by which we may say definitely that an isolated cell is a cancer-cell or a normal cell," both indicate that the problem of single cell diagnosis of cancer is still to be settled. Rea-

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sons for this skepticism are found in the small number of tumors dealt with in most of the reports, the great variety of tumors within the small groups, the diversity of methods used, and often the inadequate number of photomicrographs, limiting comparison of results.

Detailed descriptions of cytologic characteristics, such as have been reported in bone marrow studies [31] and more recently in investigations of lymph nodes [36,39] and spleens,[40] have not been recorded for malignant tumors. As a consequence, cytologic descriptions of tumors are limited to generalities such as anisocytosis, peculiar structure of chromatin and hyperchromasia of nucleus, size of nucleoli, or emphasis upon a detail (Quensel,[4,6,7] MacCarty [2,8-10]) which may have statistical significance but is of little help in the interpretation of a given case.

In contrast is the work of Papanicolaou[3] and his followers.[45,46,48-50,52,54] Utilizing a standardized technic and concentrating upon the cells of body excretions (vaginal, bronchial, etc.), they have demonstrated the usefulness of cytologic diagnosis of carcinomas.

From the above it appears that the most fruitful approach to this problem would be the examination of a large number of tumors, using a standardized technic and cataloging the changes which would make it possible to differentiate malignant from nonmalignant cells, regardless of origin. Such careful studies might eventually reveal differences permitting further classification, *i.e.*, carcinoma, sarcoma, or perhaps within a certain group even a detailed diagnosis, as is possible today with histologic methods (adenocarcinoma, squamous cell carcinoma, etc.).

It is in this manner that the present study was carried out. The tumors examined were limited to histologically proved carcinomas from various sites, and search for answers to the following questions guided the investigation:

1. What are the cytologic characteristics of carcinomas studied by means of the smear technic? Or, rephrased, is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue, using the cytologic method?

2. Is there a cell or cells which characterize carcinomas, and are such cells always absent in noncancerous conditions?

3. Is there any relationship between the organ from which the tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or skin?

4. Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic features? In other words, are the cells which make possible the cyto-

logic diagnosis of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

5. Is the cytologic picture (smear) of a metastatic tumor the same as that of the primary tumor?

Technic

The ideal method is to prepare smears of cells immediately upon removal of the tissue by the surgeon. Unfortunately, this was not possible in the majority of cases. Thus, the first problem was to determine what cellular changes occur following removal of tissue from the body. Since Rohr and Hafter [30] have shown that the changes in bone marrow after death are progressive, all of the tissues used in this study were refrigerated (4.0° to 5.0° C.) as soon as possible in order to retard these autolytic changes. To determine what changes the cells undergo at this temperature and what medium might best preserve them, fresh specimens were placed in four test tubes: (1) Fresh tissue in formalin, (2) fresh tissue in 0.85 per cent solution of sodium chloride, (3) fresh tissue in human plasma, and (4) fresh tissue untreated. All of the tubes were refrigerated (4.0° to 5.0° C.) and their contents examined in smears at intervals of 1 to 2, 6, 12, 24, 48, and 72 hours. It was observed that cells placed in formalin showed great changes in their staining reactions in less than 1 hour; those areas to which formalin had not penetrated (center of specimen) showed no tinctorial change. The cells in a physiologic solution of sodium chloride revealed remarkable changes within the first 12 hours—a fact noted many years ago by Forkner.[32] The nuclei became swollen, the chromatin structure assumed an irregular, wide-meshed, net-like appearance, and the nucleoli became more visible. Finally the cell disintegrated. Cells suspended in human plasma, or untreated, showed almost no changes in staining reaction, cell form, or shape up to 24 hours at refrigerator temperatures. After this, they usually changed slowly, but in a manner different from the cells suspended in physiologic solution. Because of this experience all tissues which could not be examined immediately were placed under refrigeration in human plasma or untreated.

The second problem was to discover which method would best preserve the cell form and size, and produce an even distribution of cells in the preparation. The usual methods for making smears described in the literature [1,31,36] and also touch preparations proved unsatisfactory for two reasons: (1) The cells were unevenly distributed and often were elongated in the direction of smearing; (2) tissues rich in mucus or fat (*e.g.*, mucosa of the gastro-intestinal tract, breast tissue) gave smears very poor in cells; the same was true of tumors which

contained much fibrous tissue. These methods, however, gave good results in material obtained from lymph nodes, spleen, and squamous cell carcinoma. Very poor results were obtained with the crush method [20,23,25] of forceful spreading of tissue between two slides; too many clumps of cells were present to permit uniform staining or to preserve the shape of the cells.

The following method was found to be most satisfactory with all organs and tumors. A fragment of tissue, not larger than 2 to 4 mm. in diameter, was placed on a slide and covered with 3 or 4 drops of pooled human plasma. Using two needles, the tissue was teased apart until the plasma was clouded by the suspended cells. One drop was transferred to a coverslip and then covered with a second coverslip, producing an even distribution of the cell suspension. The coverslips were then pulled apart and dried. This method is similar to that used by some hematologists [57] in the preparation of blood smears.

The third problem was the selection of a stain. It was desired to preserve the cells as well as possible and, at the same time, to differentiate clearly the various cellular structures. Most of the methods described in the literature, judging from illustrations and descriptions, did not seem to give these desired results. Usually the chromatin of the nucleus was clumped so as to prevent recognition of the more delicate structures (Martin and Ellis' [22] method with hematoxylin and eosin, Dudgeon and Barrett's [1] method with Schaudinn's solution and subsequent treatment with hematoxylin, iodine, and eosin). It was found impractical to use the more sensitive method of supravital staining described by Sabin [58] or Quensel's [4] method for examination of tumor cells, since in both cases it is necessary to use very fresh material, which was not always possible in this work. Two methods appeared to be better adapted to my purposes: The various combinations of Romanowsky stains currently in use in hematology,[31,57] which show clearly the details of cells; and Papanicolaou's [3] method, which has proved so popular in recent years in the examination of tumor cells of different body excretions (vaginal fluid, sputum, urine, gastric juice). Therefore, in this study several preparations of each specimen were stained, some with a combination of May-Grünwald-Giemsa's stain, others with Wilson's * stain, and still others by Papanicolaou's method (hematoxylin-EA_{25}-OG_6). Of the two hematologic methods, eventually only Wilson's stain, because of its simplicity, was retained, although both methods gave equally good results. The material stained by Papanicolaou's method was prepared upon slides, according to his directions.[3] In comparing the two methods (of Wilson and of Papanic-

* Phosphate buffered Wright's stain.

olaou), the details, particularly of the nucleus (chromatin structure), are more clearly differentiated by Wilson's stain, although Papanicolaou's method was sometimes more valuable for studying cell outlines. Specimens containing much fat, mucus, or colloid were likewise better stained by Papanicolaou's technic. Wilson's stain, on the other hand, had the advantage of bringing into greater contrast the nucleus (violet) and nucleolus (blue), as well as nucleus (violet) and cytoplasm (light blue to dark gray-blue). With Papanicolaou's method most of the tumor cell cytoplasm was stained green, the nucleus grayish violet. The nucleolus, although readily visible, appeared only as another variation of the same gray-violet; the chromatin was more distorted than when Wilson's stain was used. Both methods have been used to complement each other in almost every case.

Material

In accordance with the questions stated in the introduction, two classes of material were examined: Histologically proved carcinomas, and normal and noncancerous (inflammatory, benign tumor, etc.) tissues from various sites.

A total of 188 cases have been examined; 78 in the first group (carcinoma), and 110 in the second (control) group, noncancerous. From the 188 cases, 268 organs or tissues have been examined; 178 were noncancerous, 90 fell into the carcinoma group. They have been individually classified. For instance, if in a case of carcinoma of the cardia of the stomach, the tumor, the grossly unchanged mucosa of the fundus, and a mesenteric lymph node were examined, each area was separately classified as noncancerous or carcinomatous according to its histologic findings. In the same way two or more organs or tissues from a case without a carcinoma were individually examined and classified.

The noncancerous tissues are listed in Table I, and the carcinomas, according to the organ from which they were taken, in Table II. It must be emphasized that the classification in Tables I and II is based not upon cytologic, but upon histologic, examination. In this manner each organ or tissue was appropriately cataloged as either noncancerous or carcinomatous.

Results

It is not intended to describe in this paper the cytologic features of noncancerous organs or tissues in smear preparations, except in so far as it may be necessary to elucidate the differences between noncancerous processes and carcinoma. On the other hand, it is necessary to describe the cytologic characteristics of those tissues which have been classified histologically as carcinoma.

The over-all cytologic picture of the carcinoma smears shows certain characteristics which occur repeatedly. These characteristics, which may be subdivided, suggest a possible cytologic classification. Five types could be distinguished among the smears of 90 carcinomas: The squamous cell type, the columnar cell type, the round cell type, the undifferentiated cell type, and the oat cell type.

Carcinoma Smears of Squamous Cell Type (Fig. 1)

In carcinoma smears of squamous cell type, squamous cells were present in varying numbers, just as in smears of normal skin or mucous membranes (Fig. 6); most of the cells, however, were atypical, varying in size, shape, and color. Instead of the usual polygonal form with

TABLE I

Tissues Histologically Classified as Noncancerous

Organ (tissue)	Number of specimens examined
Esophagus	2
Stomach (corpus and fundus, 8; pylorus, 2)	10
Duodenum	1
Jejunum	2
Ileum (no lesion, 1; inflammation, 2)	3
Colon	9
Rectum	5
Diverticulosis of intestine	1
Polyp of colon	1
Bronchus	1
Lung (no lesion, 1; lung abscess, 3)	4
Uterus (endometrium, 22; cervix (cervicitis), 26)	48
Ovary	9
Breast (chronic mastitis, 1; granulomatous lesion, 1; cystic hyperplasia, 2; fibrosing adenomatosis, 2; comedo-adenoma, 1; intracanalicular fibro-adenoma, 3; adenofibroma, 1)	11
Testis	2
Benign prostatic hypertrophy	4
Kidney (pyelonephrosis)	1
Bladder	1
Submandibular gland	1
Mixed tumor of parotid	1
Gallbladder (no lesion, 1; cholecystitis, 5)	6
Thyroid (cystic nodular goiter, 1; nontoxic adenoma, 1; lymphadenoid goiter, 1; hyperplasia and hypertrophy, 2; fetal fibro-adenoma, 1; nodular colloid goiter, 1; embryonal adenoma, 1; nodular goiter with fetal adenoma 1; colloid goiter, 2; nodular thyroid, 1; nodular goiter with hyperplasia and colloid nodules, 1)	13
Lymph node (no lesion, chronic inflammation, Boeck's sarcoid)	16
Spleen	7
Skin	2
Striated muscle	1
Fibromyoma uteri	3
Sinusitis maxillaris	1
Nonspecific inflammation	6
Epiglottis	1
Lipoma	2
Papilloma of abdominal wall	1
Vagina	1
Giant cell epulis	1
Total	178

a dense, dark, almost pyknotic nucleus and abundant cytoplasm which stains light pink or bluish, the cells often were elongated and stained darker blue, but were still pale; the nucleus sometimes was larger than is normal, and the chromatin might be net-like. These cells were readily recognized as variations from the normal squamous cell. Figures 7 and 8 depict a group of cells which showed a close relationship to the

TABLE II

Neoplasms Histologically Classified as Carcinoma Listed by Location

Location	Number of specimens examined
Lip	3
Mouth	2
Esophagus	1
Stomach	9
Colon	7
Rectum	8
Larynx	1
Lung	4
Mediastinum	1
Uterus (endometrium, 3; cervix uteri, 6; infiltrating from ovary, 1)	10
Ovary (primary, 4; metastatic, 1)	5
Vulva	2
Breast	12
Pancreas	1
Thyroid	1
Kidney	1
Bladder	1
Peritoneum (metastatic)	2
Neck	1
Lymph node (metastatic)	13
Abdominal wall (metastatic)	1
Chest wall	1
Skin	3
Total	90

squamous cells described above. Their cytoplasm was bluish, sometimes blue, almost always well outlined; rarely, one or two borders merged gradually with the background. The size of the cell and its shape were more or less the same as those of the previously mentioned cells; sometimes they were slightly larger. Elongated forms and spindle-like types were more common than in the former. The nucleus, however, was always larger, varying from the size of a large lymphocyte (8 to 10 μ) to 15 μ or more in diameter. When the nucleus was large, it formed the greater portion of the cell, so that the cytoplasm was relatively sparse. The nucleus, as a rule, was well delineated and round, rarely slightly oval. The chromatin formed a regular, medium-sized meshwork. It contained one nucleolus, rarely two or three, each 2 or 3 μ in diameter. Nucleoli were usually round and blue or bluish; exceptionally they were dark blue and slightly irregular. This cell is designated a "nucleolated squamous cell." In some instances cells were

seen which resembled those found in the deeper layers of normal epithelium (basal cells, according to Papanicolaou [3]). They usually appeared in groups; single cells were seen rarely. These cells are henceforth termed "malignant epithelial cells" (Figs. 9 and 10). They varied in size (10 to 20 μ), and were rhomboid or polygonal. The cytoplasm was dark blue and rather sparse; it was not very sharply outlined, but the border was still visible. Sometimes keratin formation, as described by some authors,[26] was seen in the cytoplasm in the form of red granules, small rods, or dust. The main mass of the cell usually was constituted by the nucleus, which was oval to round and usually slightly irregular; large forms predominated. The chromatin was granular, irregular, rather coarse, amorphous, and often was condensed in the form of a ring at the periphery of the nucleus. The nucleoli were multiple (one to four), dark blue, irregular, poorly outlined, and measured 1 to 3 μ or more in diameter. Another cell seen in smears of this type was a giant cell (Fig. 13), which always appeared singly. Exceptionally as many as three or four per low-power field might be seen. The sparse cytoplasm, which stained dark blue, usually was present only at the poles of the nucleus, disappearing gradually without sharp borders at the periphery. Sometimes the cell contained small cytoplasmic vacuoles. The nucleus was large (20 to 40 μ) and usually irregular because of many indentations. It possessed numerous nucleoli, which were blue; but, because of the presence of small granules of dense chromatin which produced a turbid appearance, the nucleoli often were difficult to see. A fibroblast-like cell (Figs. 11 and 12) was frequently seen in these smears. It usually was larger than true fibroblasts. The cytoplasm was darker, often dark gray-blue, poorly outlined and usually fringed at its margin. The nucleus was long, oval, and narrow; the chromatin was net-like, its strands sometimes thick and irregular; the nucleoli were light blue to blue. The term "pseudofibroblast" will be used to identify this cell. These cells varied in size and sometimes showed transitional forms to the above-mentioned giant cells and nucleolated squamous cells; on the other hand, it frequently was difficult to distinguish them from fibroblasts. Such cells have been described by Papanicolaou [3] and others in vaginal smears of carcinoma of the cervix. Comparing the smears with the histologic preparations led to the opinion that they probably are not fibroblasts. It is more probable that these spindle cells are derivatives of epithelium. In connection with this it should be mentioned that in experimental tumor transplantation occasionally a carcinoma will show sarcoma-like changes, that is, the appearance of spindle cells. The explanation (Ewing [59]) is that the spindle cells represent derivatives of epithelium, rather than a change in the character of the tumor (carcinoma to sar-

coma). Besides cells of the four types mentioned above, in carcinoma smears of squamous cell type were found erythrocytes, leukocytes, fibrocytes, and fibroblasts. (For their description see Tischendorf.[39])

One or another cell form, as described above, may predominate in the smear. Basically, however, the smears show a very similar appearance. Smears of this type were found in cases of carcinoma of the lip, mouth, esophagus, larynx, bronchus, cervix uteri, vulva, kidney, skin, and in some metastatic carcinomas in lymph nodes. In one case of cervical "carcinoma *in situ*," only such cells as are illustrated in Figure 10 (malignant epithelial cells) were seen.

Carcinoma Smears of Columnar Cell Type (Fig. 2)

The columnar cell (Figs. 14 and 15) in all its variations characterized this type. In its usual form it measured approximately 6 by 15 μ, possessed a round to oval nucleus, and light blue to blue cytoplasm with well demarcated outlines. Sometimes red, dust-like granules were visible at one end of the cytoplasm. Among these cells were others which had larger nuclei. These sometimes were irregular; their chromatin was arranged in strands of varying thickness. Often small, irregular nucleoli were seen. The cytoplasm of these cells was poorly defined and dark grayish blue. Another cell similar in many respects to those just described was found only in carcinomas (Fig. 16). It, too, was more or less columnar, showed the same variations as the above described cells, with the addition that usually two or three irregular, dark blue nucleoli were present. Where only one nucleolus was found, it was large and round. The nucleoli might occasionally be pale but retained all the other properties described. This cell was named the "malignant columnar cell." The third cell type, seen only in carcinomas, was the "undifferentiated cell" * (Fig. 19) with no cytoplasm or with a little cytoplasm in the form of a narrow, perinuclear, light grayish zone. The nucleus measured 12 to 20 μ in diameter. It usually was round and irregular with net-like chromatin, the strands of which were rather thick, sometimes forming wide meshes. At other times the chromatin was more granular in appearance. The nucleoli were large, pale blue, irregular, and often multiple. Large round cells were seen also in smears of this type (Fig. 17). They usually were round and relatively well delineated. The nucleus usually was about 12 to 18 μ in diameter, round, and predominantly regular. The chromatin did not form strands in most of these cells, but usually was amorphous in appearance and irregularly distributed, producing darker and lighter areas. The nucleoli were round, dark blue, and often masked by the dense chromatin, so that their borders were not clearly visible.

*The term "undifferentiated cell" is one of convenience. Whether it is cytogenetically less differentiated than the other cells is not known.

In other cases in which the chromatin formed an irregular, coarse net, the nucleoli were seen readily and were round, large (3 to 8 μ), and blue. The cytoplasm was bluish or blue and well preserved in many of these cells; in some, however, its margins faded gradually into the background. These cells, examined under low power, frequently resembled plasma cells, at first glance. The large, round cells might achieve considerable size so that they formed giant cells (Fig. 18) comparable in magnitude to those seen in the squamous cell smear. However, the cytoplasm of these cells, in contrast to that of the giant cells of the squamous cell smear, surrounded the nucleus and was not localized largely at the poles. This appearance suggested that the cells were derived from the large round cells. Giant cells, as illustrated in Figure 13 and described in connection with smears of the squamous cell type, were seen also in the smears of the columnar type. The same was true of the pseudofibroblast (Fig. 12). Both of these forms were rare in smears of this type, as compared to those of the squamous cell type. In addition to the above-mentioned cells, leukocytes, erythrocytes, fibroblasts, fibrocytes, and macrophages were present.

Carcinoma smears of the columnar cell type have been found in carcinomas of the stomach, colon, rectum, and ovary, and in some metastatic carcinomas of lymph nodes.

Carcinoma Smears of Round Cell Type (Fig. 3)

In some cases classified as carcinoma of round cell type, large, round cells (Figs. 17 and 18) composed the entire smear; in other cases some columnar cells (Fig. 16) were present. The impression, in the latter event, might be that of a smear of the columnar type with predominance of large, round cells. In other instances no columnar cells could be seen. In addition, a few undifferentiated cells might be visible.

Smears of this type have been seen chiefly from carcinomas of the cardia of the stomach and in some carcinomas of the breast, lung, pancreas, bladder, and uterus. This type was seen twice in smears of metastatic carcinoma.

Carcinoma Smears of Undifferentiated Cell Type (Fig. 4)

What has been said of smears of the round cell type may also be repeated in a description of the undifferentiated cell type, with the difference that the predominating cell was that previously described as the "undifferentiated cell" (Fig. 19). Smears which revealed such a picture have been obtained from carcinomas of the stomach, colon, rectum, ovary, and breast.

Carcinoma Smears of Oat Cell Type (Fig. 5)

The word "oat" is used for this type because the characterizing cells resembled those found in the sputum of patients with carcinoma of the respiratory tract and described as oat cells. Two forms of small cells were seen in this type. One represented cells which could not be differentiated from those of normal organs or tissues. The nucleus was the size of that of a small to large lymphocyte with little variation in size and shape; it was round to oval. The chromatin usually was dense, sometimes granular and irregularly distributed. Nucleoli were very rare. No cytoplasm was present, or, if present, it appeared only as a narrow bluish zone about the nucleus. The cells usually appeared in large sheets and might be compared to the type which Rohr and Hegglin [20] have designated "kleinzelliger Carcinom Typ" in bone marrow. There appears to be justification for their opinion that a diagnosis may not be made on the basis of a single cell, but only from cells which occur in the form of sheets. Whether the latter is true in every case is, in my opinion, open to question, because in some smears of chronically inflamed spleens and lymph nodes similar cells have been found, often in small groups.

The second small cell is that described in the literature [16] as the oat cell of bronchial carcinoma. It was similar to the first small cell, except that it possessed a definite but narrow rim of blue cytoplasm. The cytoplasm formed a short bipolar process. This cell likewise appeared frequently in sheets, but might sometimes be recognized when isolated. Finally it should be mentioned that, just as in the smears of the first two types, other cells were present: erythrocytes, leukocytes, fibroblasts, fibrocytes, and macrophages.

Smears of this type were seen in one case of carcinoma of the thyroid, in a case of pulmonary carcinoma, and in almost half of the proved metastatic carcinomas in lymph nodes.

Table III presents the different types of smears and the histologic classification of the carcinomas from which the smears were made.

Discussion

1. *What are the cytologic characteristics of carcinomas studied by means of the smear technic? Is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue using the cytologic method of study?*

The most widespread and accepted method of cancer diagnosis—histologic study of tissue—is based upon the recognition of several changes in structure, many of which are cytologic: increase in the number of cells, marked variation in their size and shape differing from

the original cell type, increase of the chromatin mass, large nucleoli, multiple and abnormal mitotic figures, loss of polarity, and infiltration of the surrounding tissue (Aschoff,[60] Ewing,[59] MacCallum[56]). All may be studied by cytologic methods except the last, which is often considered the most important. Since cytologic technics preserve the components of a cell in greater detail than the methods of histology, vari-

TABLE III

Types of Carcinoma Smears with Corresponding Histologic Classification

Type of smear	Histologic classification
Squamous cell type, 29	Squamous cell carcinoma (lip, 3; mouth, 2; esophagus, 1; larynx, 1; lung, 1; cervix uteri, 4; vulva, 2; lymph nodes, 5; skin, 2) Carcinoma (bronchogenic, 2; kidney, 1; neck, 1; cervix uteri (*in situ*), 1) Tissue with degenerating neoplastic(?) cells (skin, 1) Benign* (chronic cervicitis, 1; cervix with no lesion, 1)
Columnar cell type, 22	Adenocarcinoma (stomach, 4; colon, 5; rectum, 6; endometrium, 1; uterus, 1; ovary, 2; lymph node, 2) Malignant adenoma (uterus, 1)
Round cell type, 13	Adenocarcinoma (stomach, 3; cervix uteri, 1; endometrium, 1; lymph node, 1) Carcinoma (lung, 1; pancreas, 1; bladder, 1; mediastinum, 1; duct cell of breast, 1; canalicular of breast, ; medullary of breast, 1)
Undifferentiated cell type, 14	Adenocarcinoma (stomach, 2; rectum, 1; colon, 1; peritoneum, 2; abdominal wall, 1; ovary, 3) Carcinoma (rectum, 1; chest wall, 1; scirrhous of breast, 1; medullary of breast, 1)
Oat cell type, 9	Adenocarcinoma (thyroid, 1) Carcinoma (colon, 1; lymph node, 5) Benign* (nodular hyperplasia and fibrosing adenomatosis of breast, 1; cystic hyperplasia of breast, 1)
No finding, 4	Carcinoma (canalicular of breast, 2; scirrhous of breast, 2)

*These cases will receive further comment.

ous cytologists from time to time have selected one or more of the above characteristics as their criteria for cancerous changes.

The opinion popular among older authors (literature by Quensel[7]) was that cancerous cells are larger than normal cells, and that the ratio of nucleus to cytoplasm is changed in favor of the former. This observation also has been made more recently,[3,38] but the change is considered suggestive, rather than characteristic, of malignancy. My smears showed that the majority of carcinomas do have larger cells than those of the tissue from which they arose. It appeared impossible, however, to make a diagnosis of malignancy on the basis of the size of a cell or the ratio of nucleus to cytoplasm.

Quensel [4,6,7] studied malignant cells in body fluids using the method of supravital staining. He stated that the nucleolus is of diagnostic importance. In his cases of carcinoma the nucleoli varied in diameter from 3 to 10 μ; exceptionally they were smaller, 1.5 to 2 μ. In contrast, the nucleoli of endothelial cells (control) measured 1 to 1.5 μ, very occasionally 2 to 3 μ. He showed that the size of the nucleolus in carcinoma is independent of that of its nucleus. This is indicated by the ratio, nucleolus:nucleus, which is 0.20 to 0.60 in carcinoma, as compared to 0.14 to 0.20 or less in endothelial or other cells. Further characteristics of malignancy, which Quensel considered less important, are giant vacuoles (larger than 40 μ) and irregular sheet formation with partial or total overlapping of nuclei, as compared to other cells in body fluids in which the vacuoles are smaller and the cells form "plaques." The change in the size of nucleoli was not found in sarcomas. Quensel's results were confirmed by Zadek and Karp [61] and Zadek,[62,63] who studied tumors of pleura, peritoneum, lung, and urogenital tract. MacCarty and his co-workers,[2,8-10,13] using a different method, arrived at similar results showing that the ratio of the nucleolar to nuclear surface, estimated as an average from a large number of cells (25 to 100), was always greater in malignant processes than in any other lesions of a particular organ (tissue). This ratio varied from 1:5 to 1:17 in malignancy and from 1:13 to 1:45 in control cases; they considered this ratio to be of diagnostic value. Rohr and Hegglin [29] showed that in the bone marrow only a certain number of carcinomas possess the cell characteristics which Quensel ascribed to them. Other carcinoma cells may have small nucleoli or even none. Thus, while it may be that a large nucleolus or a certain ratio of the nuclear diameter (surface) to that of the nucleolus may be characteristic of carcinoma cells in body fluids, it is not necessarily true for carcinoma cells in general. Similar observations have been made on my material. Quensel and MacCarty's observations seem applicable to a large number of my carcinomas (stomach, breast, and endometrium). Many others, however (lung, thyroid, and some of the breast carcinomas) did not fit into this scheme. The cells of metastatic tumors often had only small nucleoli or none.

Irregularity of cell shape and size, changes in the structure of chromatin, and atypical staining reactions of cytoplasm have been stressed by almost every author who has studied malignant tumors cytologically, either directly from the tumor or in excretions and body fluids. These changes have not been considered diagnostic, but only suggestive of malignancy. Similar changes can be seen in inflammatory lesions as well as in benign tumors, usually, however, to a lesser extent. The

same opinion may be expressed concerning irregular sheet formation with overlapping of nuclei. While this phenomenon is common in carcinoma, it can be seen also in other processes (inflammation). In summary, therefore, it may be said that many cellular changes suggest malignancy but are neither sufficiently constant nor sufficiently prominent to establish their diagnostic significance. When seen in a smear, they should, however, awaken the suspicion of the observer.

These suggestive changes as seen in my material are summarized in Table IV. As may be seen in this table, which is based on the study of smears from 90 carcinomas, there were certain cellular changes suggestive of a process which may not be benign. The size of the individual *cell* in carcinoma smears was, in general, larger than that in noncancerous processes; this was especially noticeable when the cells were compared with those of the tissue from which the carcinoma arose. Variation in size and shape might often be very marked, although some tumors showed a relatively uniform cell picture (instances of carcinoma of bronchus, carcinoma of thyroid). The cells were individually disposed or were present in sheets. When in the latter form, the nuclei were partially superimposed and polarity was lost to a greater or lesser degree, as compared with noncancerous processes, in which the "plaques" consisted of a single layer of cells, the nuclei of which were regularly arranged. There were, however, noncancerous processes which might show the same phenomena as carcinoma, but to a lesser extent. The adhesion of cells to form groups of various sizes and shapes was much more commonly seen in noncancerous epithelium than in carcinomas, in which most of the cells usually lay separated from each other.

The *cytoplasm* of the cells in carcinoma smears was either scanty or poorly outlined, merging gradually with the background. Few of the carcinomas possessed cells which were relatively rich in cytoplasm (nucleolated squamous cells, Fig. 7; some of the malignant columnar cells, Fig. 16). The cytoplasm varied from light grayish pink to blue and often dark gray-blue. Noncancerous cells usually were light blue to blue and, in addition, had more abundant and better outlined cytoplasm.

The same morphologic variations which were described in carcinoma cells, in general, can be said to characterize the *nucleus*. It was usually larger than that of the control group and sometimes attained giant dimensions (Figs. 13 and 18). It was round to slightly oval, often irregular, in contrast to the nucleus of noncancerous tissue, which was more regular. Anisonucleosis and poikilonucleosis were almost always present in the cytologic picture of carcinoma, rarely in the control

TABLE IV

Changes Suggestive of Malignancy Seen in the Smears of 90 Carcinomas as Compared with Those of 178 Control Tissues

	Findings based on examination of single cells		Findings based on examination of groups of cells	
	Noncancerous tissue	Carcinoma	Noncancerous tissue	Carcinoma
Cell	Regular in size and form; color light gray to blue	Irregular (anisocytosis and poikilocytosis); larger than the original tissue cell; color often dark gray-blue	Uniform appearance; in rows or "plaques"	Anisocytosis, poikilocytosis; in clumps and large sheets
Cytoplasm	Usually well outlined; moderate to abundant in amount	Poorly outlined; rarely well limited; often nuclei without cytoplasm; commonly little cytoplasm		
Nucleus	Round, oval, kidney-shaped; usually less than 15 μ in diameter	Round or oval, often irregular; usually 15 μ or more	Nuclei show regular arrangement	Nuclei cover each other and show loss of polarity; anisonucleosis and poikilonucleosis
Chromatin	Dense, clumped, or net-like, but regular	Irregular, coarse, net-like; irregular, granular; dusty; chromatin often forms a clearly visible ring at the border of the nucleus		
Nucleolus	None, or small to medium size, regular in shape; light blue or violet; rarely dark	Medium size to large or giant, often irregular; light blue to dark grayish blue		
Ratio of cytoplasm: nucleus	In favor of cytoplasm	Usually in favor of nucleus		
Ratio of nucleolus: nucleus		High		

group. When, therefore, a high degree of irregularity was present in a noncancerous process and a low degree in a carcinoma, they were indistinguishable on this basis.

The *chromatin* in the control group was dense and clumped; if net-like, it was regular and delicate. In carcinomas the chromatin was irregular, was composed of coarse strands, and formed a net-like structure. This was sometimes small-meshed, and at other times showed large, irregular meshes; both forms might be present in the same smear. In still other cases the chromatin formed a grid of coarse strands or consisted of irregular, coarse granules; it might have a dense, irregular, amorphous appearance. At times the chromatin was condensed in the form of a ring at the periphery of the nucleus.

The *nucleolus,* considered by many authors to be one of the most suggestive or even diagnostic indices of malignancy, particularly in carcinoma, was not visible in the majority of the cells of the control group. When present, it usually was not larger than 1 to 2 μ in diameter. It was regular and stained light blue or the same color as the chromatin. Exceptions, however, were found: in some of my specimens, identified histologically as progestational endometrium, the nucleoli were large and sometimes stained dark blue; the same was noted in some examples of atrophic endometrium, in tubular epithelium of a case of chronic pyelonephritis, and in the mucosal cells of a bladder with carcinoma. The smear from the last mentioned was obtained from an area reported histologically as showing "no lesion." The nucleolus of carcinoma cells assumed very peculiar forms (Text-Fig. 1). It might

Text-Figure 1. Appearance of nucleoli in malignant cells.

attain giant proportions up to the size of a small lymphocyte (6 μ) and occupy most of the nuclear mass (c). Nucleoli might be multiple, and were then usually irregular (a, d). The color might be light blue (a, b, c), as seen in noncancerous material, or dark grayish blue (d, e, f). Sometimes the outlines were indistinct and the nucleolar substance merged into the surrounding chromatin (f). At other times the nucleoli might be partially hidden in the mass of chromatin (giant cells, large round cells).

The *nucleus-cytoplasm ratio,* in my smear material, has often aroused the suspicion that carcinoma might be present, namely, when the mass of the nucleus was obviously larger in proportion to cytoplasmic mass than in the control cells. However, as already indicated, this cannot be considered a rule. The same may be said of the ratio of the nucleolar surface and/or diameter to that of the *nucleus,* which in carcinoma is usually much greater than in the control group. Zadek [61,62] stated that the nucleolus-nucleus ratio is 1:4 to 1:20 in carcinoma cells in body fluids, compared to 1:25 to 1:100 in endothelial cells. MacCarty claimed that by his method the ratio is 1:5 to 1:17 in malignant processes, as against 1:13 to 1:45 in noncancerous processes. Since my method (smear) is different from that of Zadek (supravital stain) and of MacCarty (sections of fresh tissue), it is impossible to confirm or deny their conclusions.

Many of the changes mentioned above are seen also in histologic sections and are constantly utilized by pathologists. The smear technic permits better preservation of cytologic details and therefore more exact observation of minute structures. It must again be stressed that all of the features described above are suggestive, rather than diagnostic, of carcinoma. Extreme variations, however, may eventually prove to be diagnostic.

2. *Is there a cell, or are there cells, which characterize carcinomas, and are they always absent in noncancerous conditions?*

If malignant tumors express their abnormality not only in tissue structure (irregularity, infiltration), but also in cell structure, there arise two possibilities of recognizing cancer by its isolated cells: All of the cells of a tumor may have morphologic characteristics indicative of malignancy; during the development of a malignant tumor some of the cells may possess or acquire morphologic peculiarities as a consequence of abnormal function. It is these "peculiar" cells which would appear to be of importance in the recognition of cancer.

Smears of five different types, as previously described, were found in my material of 90 carcinomas, which were obtained from various organs and parts of the body and represented diverse histologic categories (adenocarcinoma, squamous cell carcinoma, comedo-carcinoma). A number of atypical cells were seen which were not found in smears of the control group,* consisting of 178 specimens from normal organs, inflamed tissues, and benign tumors. The atypical cells, listed below, were constantly present in the carcinoma smears of appropriate type:

(a) The nucleolated squamous cell (Figs. 7 and 8)

* Four exceptions to this statement are described in succeeding paragraphs.

(b) The malignant giant cell (Fig. 13)
(c) The malignant epithelial cell resembling basal cells (Figs. 9 and 10)
(d) The pseudofibroblast (Figs. 11 and 12)
(e) The malignant columnar cell (Fig. 16)
(f) The undifferentiated cell (Fig. 19)
(g) The large round cell (Figs. 17 and 18)
(h) The oat cell (Fig. 5), only if in large sheets

These cells have been described above; the names have been selected on the basis of the most impressive feature of each. Cell *d* (pseudofibroblast) is not always distinguishable from fibroblasts; cell *h* (oat cell) is usually recognizable only when in sheets.

In some of the types of smears one of the above listed cells may predominate, or may be so numerous as to give the impression of being the only form present. This is usually the case in smears of the undifferentiated and round cell types. In other smears more than one kind of atypical cell was seen, as in the squamous cell type (nucleolated squamous cell, pseudofibroblast, giant cell), and in the columnar cell type (malignant columnar cell, large round cell, undifferentiated cell). Besides these atypical cells, in the majority of smears the proper cells of the organ in which the carcinoma was situated were seen also. In many cases it seemed possible to trace transitional forms from the atypical cells seen only in carcinoma smears to the epithelial cells of the control group. It should again be stressed that the atypical forms (*a* to *h*) are not considered to be the only malignant cells, since in smears of histologically proved metastases cells were seen which could not be distinguished from those found in inflammatory or other noncancerous processes. Another example of this observation is the smear of the oat cell type, in which often no cells were present which could not be found in the control group, and only the abnormal location and arrangement of the cells made probable the diagnosis of carcinoma. The assumption that a pathologic process may manifest itself in function only, without morphologic expression, would explain this finding.

Because previous workers [2,4,61] frequently have sought a common denominator among the structures of cells obtained from malignant tumors, an attempt was made to find such factors in atypical cells (*a* to *h*) (size of nucleolus, giant vacuoles, ratio of nucleolus to nucleus). None had general application. The cells (*a* to *h*) as units differ from the cells of the control group, but not in their parts. The possibility may not be abandoned, however, that in some tumors one detail of the cell may be sufficiently constant and characteristic to

establish its diagnostic value. Quensel [4] and others [2,61] made such claims for the nucleolus of carcinoma cells in body fluids. The general application of such a rule does not seem to me to be justified.

For the 188 cases examined, there was agreement between the histologic diagnosis and the conclusion reached from the examination of smears in 179 cases. In the remaining 9 cases the conclusion reached from smears was apparently falsely positive in 5 instances and falsely negative in 4. These 9 cases are summarized in the following paragraphs.

A. Apparently False Positives

1. Case 33 was a 35-year-old female considered clinically to have a carcinoma (stage I) of the cervix. Histologic examination did not confirm the diagnosis, but showed a chronic cervicitis. The smear revealed a squamous cell carcinomatous type.

2. Case 37 was a 34-year-old female with a clinical diagnosis of menorrhagia. Sections removed from the corpus uteri showed progestational endometrium; from the cervix, no lesions. Smears of the cervix revealed some nucleolated squamous cells with a more grayish violet cytoplasm than is usually seen in this cell form. No other cells of types *a* to *h* were seen.

3. Case 172 was a 66-year-old male with a clinical diagnosis of carcinoma of the face (temporal area). The pathologist reported: "Here and there in the dermis are isolated atypical cells with hyperchromatic nuclei that possibly are degenerating neoplastic elements, but of this we cannot be certain." The smear showed cells of characteristic squamous cell carcinoma type.

4. Case 185 was a 26-year-old female with a questionable clinical diagnosis of carcinoma of the breast. Sections taken for biopsy showed cystic hyperplasia of the breast; the smear showed the oat cell type.

5. Case 188 was a 20-year-old female with the clinical diagnosis of questionable benign tumor of the breast. Sections taken for biopsy showed lobular hyperplasia and fibrosing adenomatosis of the breast. The smears showed again the oat cell type and some giant cells, as illustrated in Figure 13.

B. Apparently False Negatives

1. Case 78 was a 46-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis was canalicular carcinoma of breast. The smear made from tissue in the neighborhood of the removed tumor could not be evaluated because of changes due to the antiseptic agent used in that area. Another smear taken from the periphery of the breast was negative for cells *a* to *h*.

2. Case 103 was a 45-year-old female with the questionable clinical diagnosis of carcinoma of the breast. The sections showed comedo-carcinoma of breast. The smear taken from the neighborhood of the tumor was negative for cells *a* to *h*.

3. Case 127 was a 43-year-old female. The clinical diagnosis was breast tumor; the histologic diagnosis, scirrhous carcinoma. The smear was negative.

4. Case 215 was a 57-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis, scirrhous carcinoma. The smear taken from the depth of the tumor showed only different forms of epithelial and connective tissue cells.

In the group of apparently false positive diagnoses, cases 33 and 172 presented the characteristic squamous cell type, despite the absence of carcinoma in the histologic preparations. I cannot explain this dis-

crepancy. Some investigators [3,53] using Papanicolaou's method on vaginal smears have noted false positives similar to mine. The correctness of one or the other finding can be determined only by observation of the patient's future course. Cases 37, 185, and 188 showed only a few cells of types *a* to *h*.

In the second group of apparently false negative diagnoses, the failure to detect tumor cells in the smears from cases 78 and 103 is not significant, since the tissue was taken from the neighborhood of the tumor rather than from the tumor itself. Although the possibility of failure in these cases was realized, the smear was taken nevertheless, since in some cases tumor cells were detected far from the neoplastic center and from locations which the pathologist reported as containing "no lesion" (Fig. 20). Cases 127 and 215 must be considered failures. It is of interest that most instances of disagreement between histologic diagnosis and cytologic findings occurred in breast tissue (6 of 9). A possible reason, at least for the negative cytologic findings, is the large amount of fat present, which makes it more difficult to obtain satisfactory smears.

In summary, specimens from 268 regions were examined from the 188 cases. In 9 instances the cytologic and histologic findings were in apparent disagreement. One or another form of atypical cell (*a* to *h*) was found in the smears of 86 of 90 histologically recognized carcinomas. Usually more than one cell form appeared in the individual smear. Similar atypical cells have been found also in 4 cases classified histologically as noncancerous. In the smears of 174 of 178 specimens histologically classified as noncancerous (no lesion, inflammation, benign tumor) the search for cells *a* to *h* gave negative results.

3. *Is there any relationship between the organ from which a tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or the skin?*

This material indicates that the organ within which a carcinoma arises exerts little influence upon the type of smear from that carcinoma. However, as has already been shown (Table III), smears of certain types are seen more frequently in certain organs than in others. This is probably dependent upon the type of epithelium which exists in that particular organ. Thus, the squamous cell type is met commonly in neoplasms of organs which normally possess squamous epithelium; the columnar cell type predominates in neoplasms of the mucosa of the gastro-intestinal tract. This is shown in Table V.

4. *Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic*

character in smears? Are the cells which make possible the cytologic recognition of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

Table VI shows the relationship between the histologic structure of carcinomas and the types of smears obtained from them.

As may be seen, the type of the smear is related to the histologic

TABLE V

Relationship Between Site of Carcinoma and Type of Smear

Organ	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Lip	3						3
Mouth	2						2
Esophagus	1						1
Stomach		4	3	2			9
Colon		5		1	1		7
Rectum		6		2			8
Larynx	1						1
Lung	3		1				4
Mediastinum			1				1
Uterus	5(2)*	3	2				10(2)
Ovary		2		3			5
Vulva	2						2
Breast			4	4	(2)	4	12(2)
Pancreas			1				1
Thyroid					1		1
Kidney	1						1
Bladder			1				1
Peritoneum				2			2
Neck	1						1
Lymph node	5	2	1		5		13
Abdominal wall				1			1
Chest wall				1			1
Skin	3						3
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

classification. All of the squamous cell carcinomas yielded smears of squamous cell type. The adenocarcinomas, however, are distributed among the columnar, round, undifferentiated, and oat cell types with predominance of the first three. The group classified histologically only as "carcinoma" produced smears of different types, since it embraced cases of carcinoma whose histologic appearance did not permit a more detailed classification. The special forms of breast tumors (duct carcinoma and canalicular carcinoma) fall into the round cell and undifferentiated types.

5. *Is the cytologic picture (type of smear) of a metastatic tumor the same as that of the primary tumor?*

It may be seen (Table VII) that of 17 examples of metastatic carci-

noma, 6 retained the original pattern in the metastases, 5 changed into the oat cell type of smear, whereas for the remaining 6 no conclusions could be drawn because the primary carcinoma was not available for study. As may be noted, when a change occurred in the metastases, it was always to the oat cell type, regardless of the type represented by the primary tumor. This change was seen only in metastases in lymph nodes. The results, although too few in number to justify conclusions,

TABLE VI

Relationship Between Histologic Diagnosis and Type of Smear

Histologic diagnosis	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Squamous cell carcinoma	20						20
Adenocarcinoma		21	7	10	3		41
Adenoma, malignant		1					1
Carcinoma (without classification)	7		4	4	4		19
Duct cell carcinoma			1				1
Canalicular carcinoma			1			2	3
Medullary carcinoma			1	1			2
Scirrhous carcinoma				1		2	3
Benign	(2)*				(2)		(4)
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

TABLE VII

Comparison of Cytologic Findings in Primary Carcinomas and Their Metastases

Organ	Primary tumor		Metastases	
	Histologic diagnosis	Type of smear	Organ	Type of smear
Mouth	Squamous cell carcinoma		Lymph node	Squamous
Stomach	Adenocarcinoma	Undifferentiated	Lymph node	Oat cell
Stomach	Adenocarcinoma	Columnar	Lymph node	Columnar
Stomach	Adenocarcinoma	Round	Mediastinum	Round
Colon	Adenocarcinoma	Columnar	Lymph node	Columnar
Colon	Adenocarcinoma		Ovary	Columnar
Rectum	Adenocarcinoma	Columnar	Lymph node	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (1)	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (2)	Oat cell
Lung	Carcinoma	Round	Lymph node	Round
Ovary	Adenocarcinoma		Uterus	Columnar
Ovary	Adenocarcinoma	Undifferentiated	Peritoneum	Undifferentiated
Breast	Carcinoma	Undifferentiated	Lymph node	Oat cell
Skin	Carcinoma		Lymph node	Squamous
Neck	Squamous	Squamous	Lymph node	Squamous
Scalp	Squamous		Lymph node	Squamous
			Lymph node	Squamous

have been discussed because of the relative lack of attention given to this finding in the literature (Naidu [13]).

Conclusions

Ninety carcinomas were studied cytologically, utilizing both Wilson's and Papanicolaou's stains. The smears were made directly from the tumors and were compared to similar preparations from 178 noncancerous tissues.

Eight cell types were recognized as characteristic of carcinoma and were found in 86 of the 90 histologically proved cancers. Similar cells were found in only 5 of the 178 noncancerous controls.

A larger number of cases must be studied before the validity of other cytologic expressions of carcinoma may be considered as proved.

I wish to express my sincere appreciation to Dr. Wiley D. Forbus for the opportunity to carry out this work, and to Dr. Bernard Black-Schaffer for his many helpful suggestions and criticisms of it.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

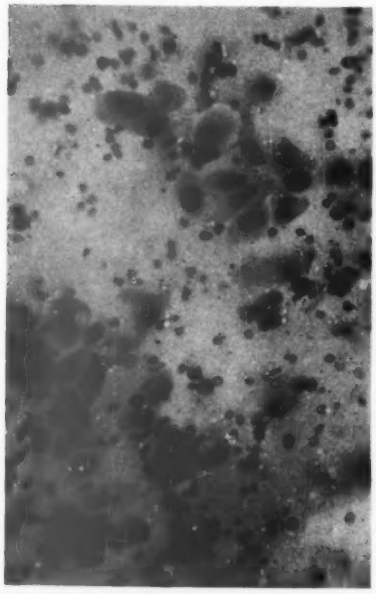
PLATE 197

FIG. 1. Case 148. Bronchogenic carcinoma; smear of squamous cell type. Wilson's stain. × 137.

FIG. 2. Case 52a. Adenocarcinoma of rectum; smear of columnar cell type. Wilson's stain. × 295.

FIG. 3. Case 21. Ductus cell carcinoma of mammary gland; smear of round cell type. Wilson's stain. × 137.

FIG. 4. Case 150. Adenocarcinoma of ovary; smear of undifferentiated cell type. Wilson's stain. × 295.



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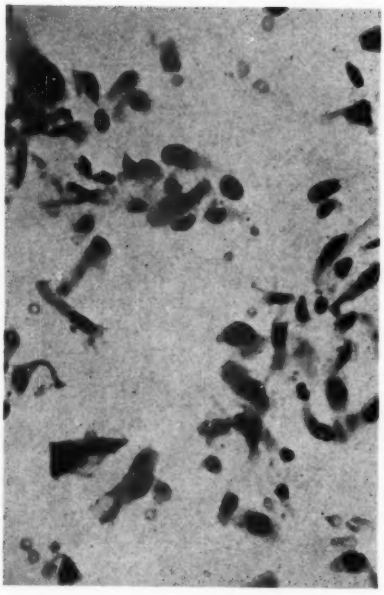

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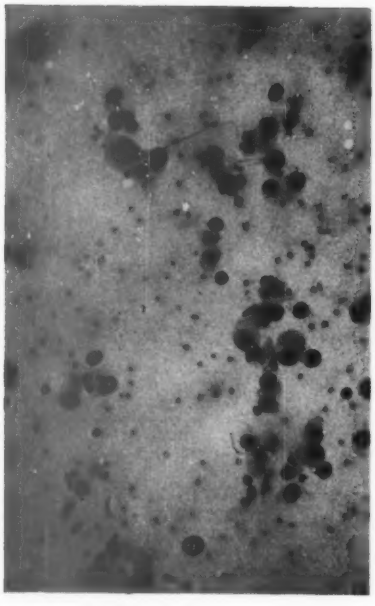

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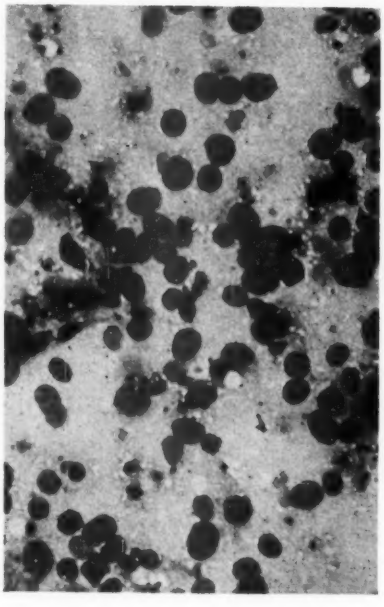

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PLATE 198

FIG. 5. Case 110c. Adenocarcinoma of thyroid; smear of oat cell type. Wilson's stain. × 295.

FIG. 6. Case 75a. Chronic cervicitis; squamous cells. Wilson's stain. × 684.

FIG. 7. Case 111a. Squamous cell carcinoma of cervix uteri; nucleolated squamous cells. Wilson's stain. × 684.

FIG. 8. Case 148. Bronchogenic carcinoma; nucleolated squamous cells. Wilson's stain. × 684.

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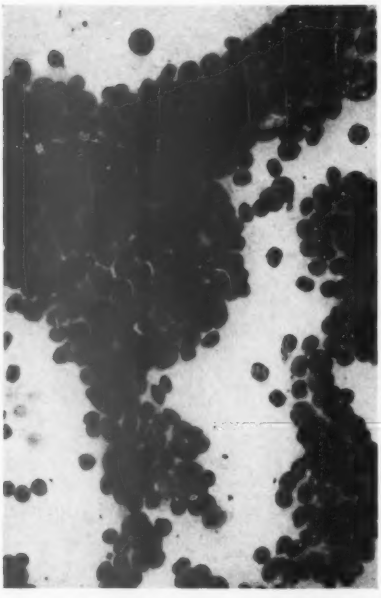

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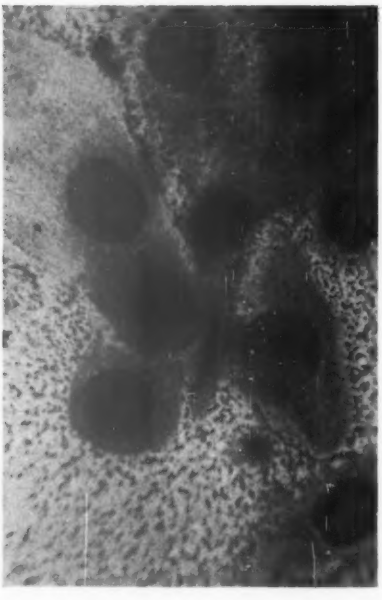

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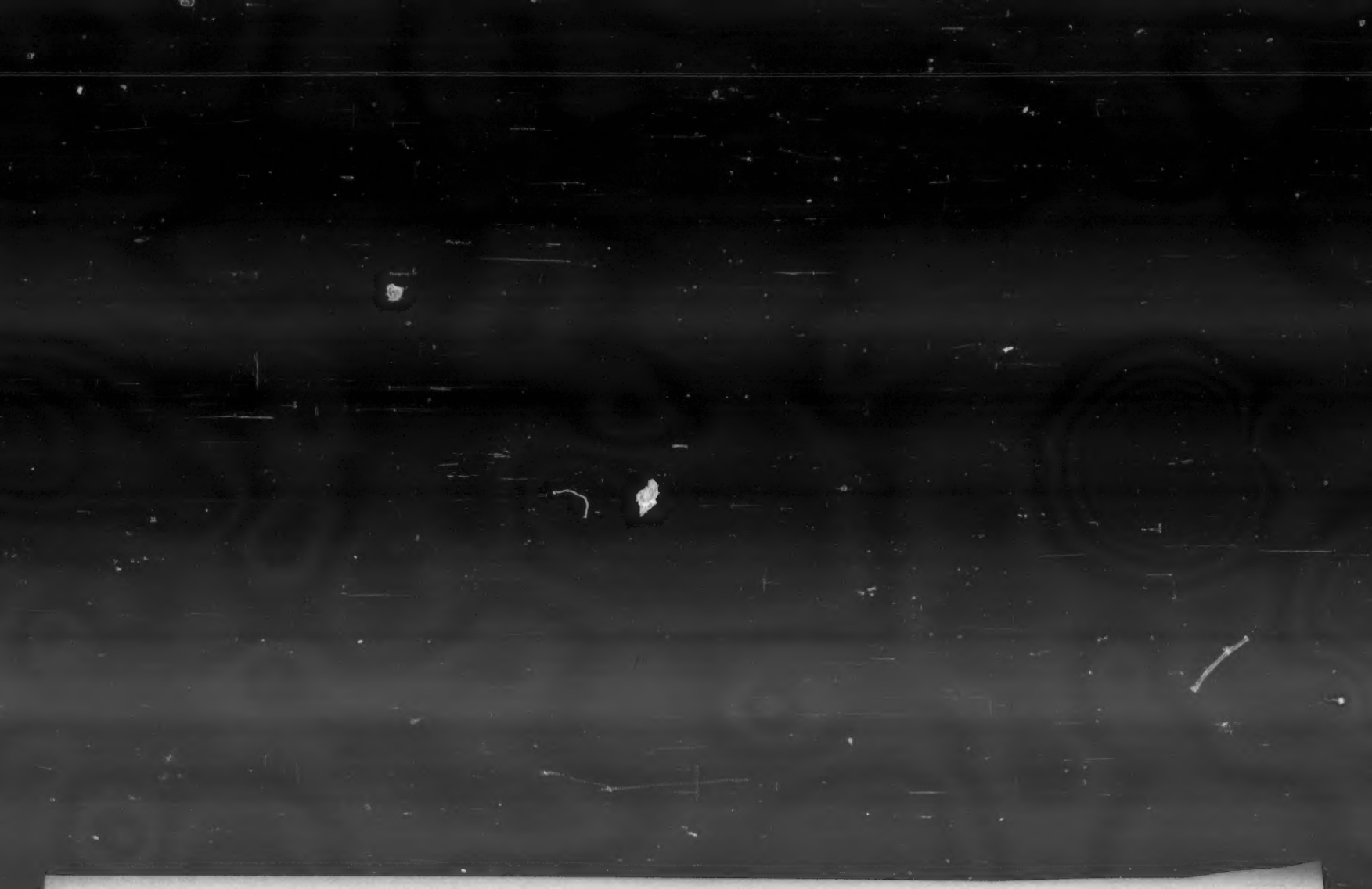
PLATE 199

FIG. 9. Case 58. Squamous cell carcinoma of vulva; malignant epithelial cells. Wilson's stain. × 684.

FIG. 10. Case 11. Carcinoma *in situ* of cervix uteri; malignant epithelial cells. Wilson's stain. × 684.

FIG. 11. Case 158b. Squamous cell carcinoma of esophagus; pseudofibroblasts. Wilson's stain. × 295.

FIG. 12. Case 111a. Squamous cell carcinoma of cervix uteri; pseudofibroblast between two other cells, one in mitosis. Wilson's stain. × 684.



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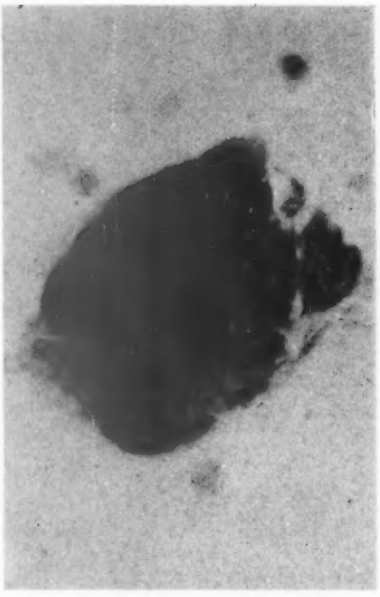

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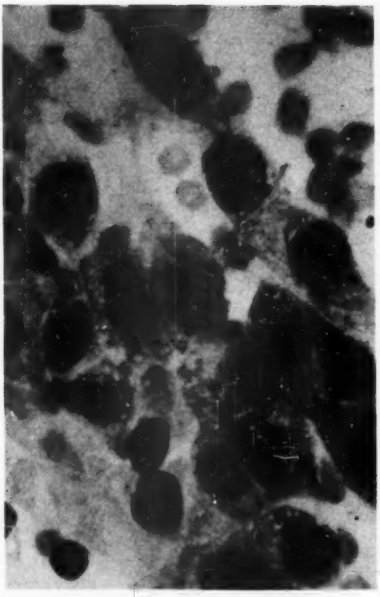

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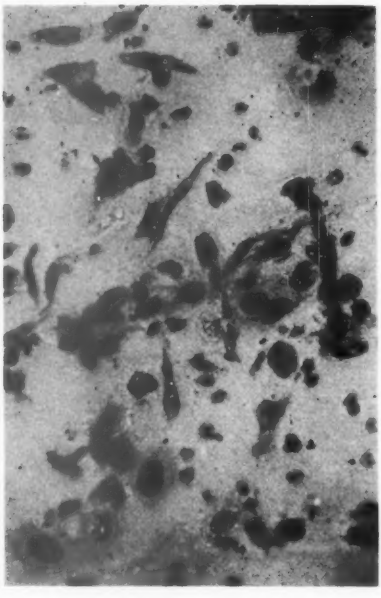

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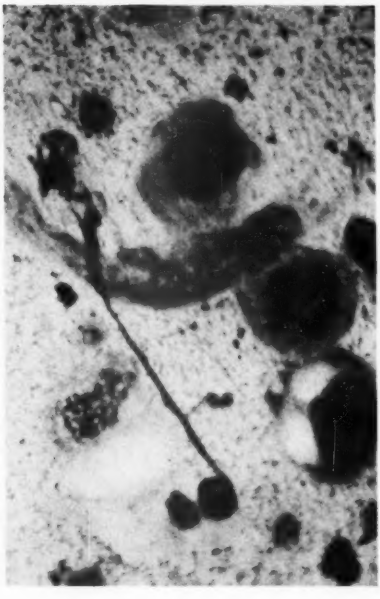

PLATE 200

FIG. 13. Case 119. Squamous cell carcinoma of lip; malignant giant cell. Wilson's stain. × 684.

FIG. 14. Case 24a. Chronic granulomatous inflammation of lung; columnar cells from bronchial epithelium. Wilson's stain. × 684.

FIG. 15. Case 140a. Chronic cervicitis and endocervicitis. columnar cells. Wilson's stain. × 684.

FIG. 16. Case 52a. Adenocarcinoma of rectum; malignant columnar cells. Wilson's stain. × 684.

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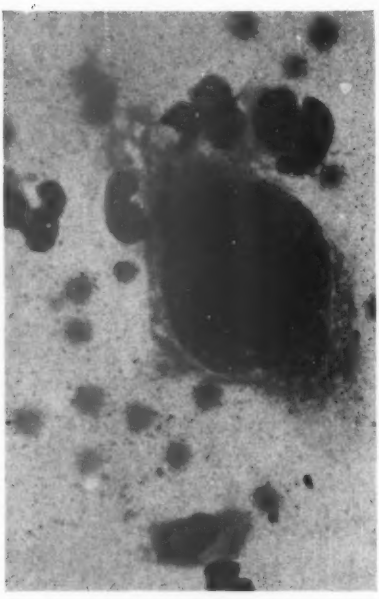

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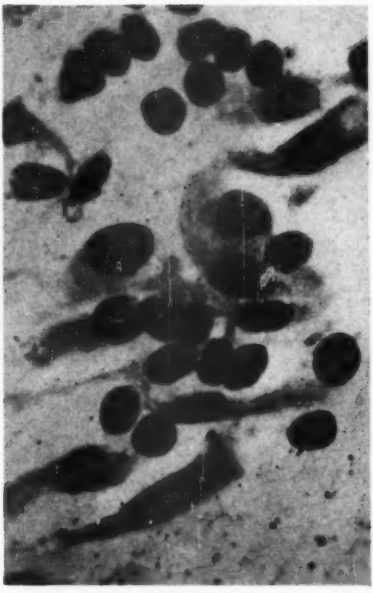

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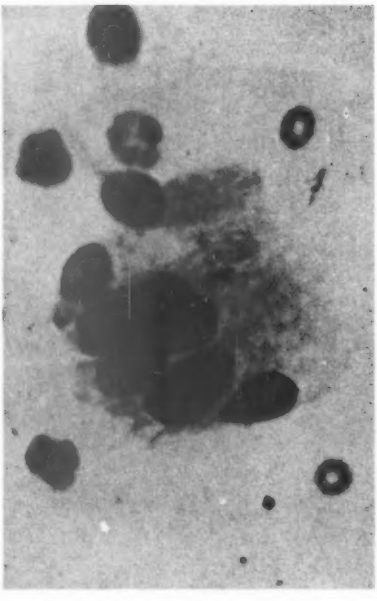

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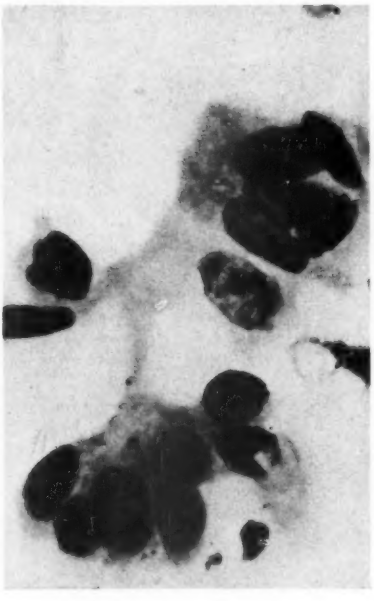

PLATE 201

FIG. 17. Case 21. Duct cell carcinoma of mammary gland; large round cells. Wilson's stain. × 684.

FIG. 18. Case 14a. Adenocarcinoma of stomach; large round cells (giant form). Wilson's stain. × 684.

FIG. 19. Case 150. Adenocarcinoma of ovary; undifferentiated cells. Wilson's stain. × 684.

FIG. 20. Case 15b. Adenocarcinoma of stomach; tumor cells in fundic mucosa, histologically classified as "no lesion." Wilson's stain. × 350.

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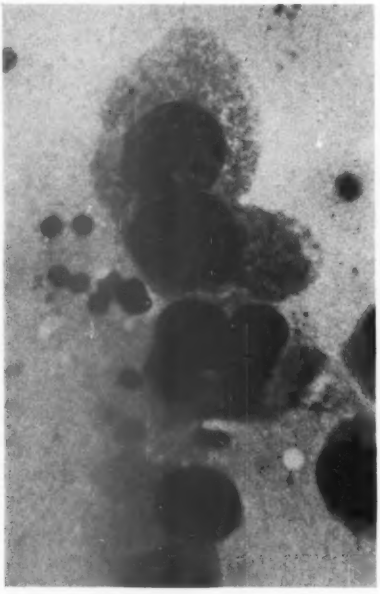

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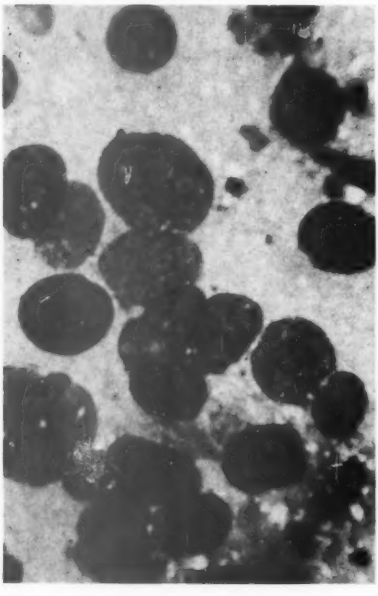

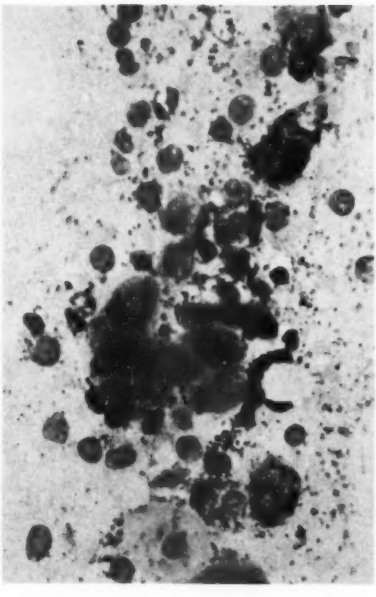

20

DIFFUSE INTERSTITIAL MYOCARDITIS IN CHILDREN *

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In recent years there have appeared in the literature reports of a diffuse interstitial myocarditis of obscure etiology. What appears to be the same pathologic entity has been variously described as isolated, primary interstitial, circumscribed, diffuse, idiopathic, acute, subacute and chronic isolated, and has been termed myocarditis perniciosa, granulomatous myocarditis, and Fiedler's myocarditis.

No attempt will be made to review the literature, since Saphir and his associates [1-3] have done this thoroughly. It is difficult to arrive at any clear definition of this diffuse type of myocarditis, but it is generally agreed that it is a nonspecific inflammatory reaction of uncertain etiology. It is rarely diagnosed clinically, and most cases remain undiagnosed until the tissues are examined microscopically. The patient exhibits progressive myocardial failure, tachycardia, cardiac enlargement, cyanosis, and low blood pressure. The course tends to be rapid and death may ensue suddenly. At autopsy, the pathologist has little to aid him in making a gross diagnosis, but usually there is evidence of congestive cardiac failure. The heart is enlarged and dilated, and the myocardium tends to be flabby and paler than is normal. Microscopic examination reveals infiltration of the myocardium by lymphocytes, large mononuclear cells, polymorphonuclear leukocytes, eosinophils, and plasma cells without involvement of the pericardium or endocardium. An inflammatory lesion of this type is more frequently seen than others, although Saphir called attention to the granulomatous type, which has appeared in case reports. Neither the gross nor the microscopic findings explain the pathologic changes found in the heart, and it is usually this organ that shows the major lesion. It should be emphasized that the histologic picture is nonspecific and similar to that seen in the diffuse myocarditis associated with various infectious diseases. Yet, as Saphir stated in his review, one is justified in accepting the occurrence of isolated myocarditis in the sense of a more or less diffuse inflammatory lesion if every known cause for this type of myocarditis is ruled out and if there is present no major pathologic condition involving either the endocardium and pericardium or the entire body.

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† Now at Dartmouth Medical School, Hanover, N.H.

A recent autopsy of an infant performed at The Children's Hospital, Boston, revealed findings which were consistent with myocarditis of this type. The cases of myocarditis at this hospital thereupon were reviewed, and among them were found 4 which seemed to fit this classification. Since most instances of diffuse interstitial myocarditis of unknown etiology have been reported in adults, it has seemed appropriate to present the reports of these in infants.

Report of Cases

Case 1

B. C., a 3-weeks-old girl, was admitted to the Infants' and Children's Hospital (no. 18940) on September 22, 1933, because of projectile vomiting, cyanosis, and dyspnea of 3 hours' duration. The family history was noncontributory. Delivery had been accomplished by high forceps. The birth weight was 10½ lb. The history of the first 3 weeks of life was uneventful. The patient had been breast-fed and was thought to be perfectly normal and healthy. Three hours before admission she awakened and vomited forcibly, and in 1½ hours she became very cyanotic, with labored respirations which continued until admission.

Physical examination revealed a well developed and well nourished female infant in extremis, with labored and rapid respirations and cyanosis. The anterior fontanelle was full but not tense, and the sutures were slightly separated. The pharynx was mildly injected. There was respiratory retraction of the costal margins. Cardiac dullness extended to the left anterior axillary border. The lungs were resonant and without râles. The abdomen was full, with the liver palpable down to the iliac crest and the spleen barely so. A roentgenogram of the chest showed marked enlargement of the heart and diffuse pulmonary congestion. The impression of the roentgenologist was congenital heart disease with passive pulmonary congestion.

The hemoglobin was 75 per cent; the red blood cell count, 4,900,000; and the white blood cell count, 32,000, with 61 per cent polymorphonuclear leukocytes, 38 per cent lymphocytes, and 1 per cent monocytes. Culture of the blood proved to be negative. Wassermann and Hinton tests of the blood of both parents gave negative reactions. A spinal fluid reaction was negative.

The infant failed to respond to therapy and died 7 hours after admission. The clinical diagnosis was congenital malformation of the heart with cardiac decompensation.

Autopsy Findings

Autopsy, performed 12 hours after death, revealed a well developed and well nourished infant with marked lividity. The abdomen was slightly protuberant and markedly tense. The peritoneal cavity contained a considerable amount of clear, yellow fluid. The liver extended 4.5 cm. below the costal margin in the midline and the spleen was just visible on the left. Each pleural cavity contained 4 cc. of clear, straw-colored fluid. The heart and lungs together weighed 130 gm. The heart was increased in size. On the anterior surface of the left ventricle and directly in the path of the descending ramus of the left coronary artery was a slightly depressed yellow-gray area of softening which measured 8 by 5 mm. There were two similar but smaller,

soft, yellow-gray areas near the left border of the left ventricle. The muscle of the apex of the left ventricle was thin. Throughout the course of both coronary arteries, the periarterial tissue was slightly raised and moderately firm. At a point 1.4 cm. above the area of softening and extending for a distance of 0.6 cm. there was a loss of pericoronary prominence, which gave an appearance of loss of contour of the artery. No congenital abnormalities were observed during complete dissection. The areas of softening in the myocardium were yellow-gray, but they did not appear to involve the pericardium or endocardium. The rest of the myocardium was slightly increased in opacity, dark pink and gray. Near the two small areas of softening there was observed a round, firm lesion, 1 mm. in diameter, which was thought by the examiner to be a thrombosed vessel. Both coronary arteries had slight tortuosity. The lungs were not remarkable except that on sectioning there was evidence of bronchopneumonia. The liver and spleen were congested and weighed 146 and 16 gm. respectively, somewhat overweight for the age. The brain was not remarkable. From cultures taken from the lung at the time of autopsy there were grown *Staphylococcus aureus, Escherichia coli,* and pneumococcus. There was no growth from the heart's blood. The impression from the gross findings was early bronchopneumonia and an enlarged heart of unknown cause.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Multiple sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, Foot's reticulum, Levaditi's, and Ziehl-Neelsen stains. Sections taken through the softened areas mentioned in the gross description revealed widespread and diffuse absence of cardiac musculature with a moderate infiltration by polymorphonuclear leukocytes, lymphocytes, plasma cells, macrophages, and large mononuclear cells. These cells had large, vesicular, oval nuclei and scanty eosinophilic cytoplasm. The majority of the muscle fibers had been replaced by young granulomatous tissue. Although there were fibroblasts present, newly ingrowing capillaries were the prominent feature. In this region remnants of cardiac muscle, without striations, and vacuolated and poorly stained, were observed. There was extravasation of red cells in many fields. The lesion was not circumscribed, but it was irregular and penetrated into areas of normal appearing muscle fibers. For the most part, the muscle tissue immediately adjacent to the inflammatory reaction showed loss of striations, vacuolation, and an occasional pyknotic nucleus. Here there was moderate interstitial infiltration by similar inflammatory cells. In one section the lesion approached the endocardium, but there was minimal

exudation. There was no definite evidence of pericarditis, although the inflammatory reaction penetrated to the pericardium. On the epicardium there was no recognizable fibrin deposition or exudate. The blood vessels throughout were dilated and congested, but there was no suggestion of arteritis. The auricular musculature in one section showed interstitial edema, but there was no interstitial lesion. Sections of the main coronary arteries did not exhibit abnormal histologic findings. Foot's reticulum stain showed no increase in the stroma in the area of greatest damage. Gram's and Giemsa's stains failed to reveal any bacteria or inclusion bodies. Ziehl-Neelsen's and Levaditi's stains were also negative. Tissue was not available for fat stains.

The microscopic sections of the other tissues were noncontributory except for moderate interstitial pneumonia, toxic splenitis, chronic passive congestion, and slight fatty change of the liver.

Case 2

L. R., a 13-months-old girl, was admitted on February 24, 1941 (no. 24543), with persistent listlessness, drowsiness, anorexia, and dyspnea of 3 weeks' duration. The family history was irrelevant except that the father was under treatment for leukemia. This was the mother's first pregnancy and it had been uneventful. The birth weight was 5 lbs., 14 oz. The baby was breast-fed for 4 days and then transferred to formula because the mother had developed pneumonia. The infant was well and very active until 5 weeks before admission, when she developed an upper respiratory infection, which apparently cleared up within a few days. The medication consisted of oily nose drops. Three weeks before admission the patient had difficulty in breathing, refused her feedings, and became listless. The family physician found no elevation of temperature and made a diagnosis of bronchitis. The patient continued to do poorly and the breathing became progressively worse, but the mother found that the baby was more comfortable when sitting up. On repeated visits by the family physician, the temperature was never elevated.

The physical examination revealed an acutely ill but well developed and well nourished female infant. The temperature was 100.6° F.; pulse, 160; respirations, 84. The pharynx and tonsils were red. There were a few enlarged cervical lymph nodes. There was dullness over the right upper chest anteriorly and in the right interscapular region. Coarse rhonchi were heard over the entire chest, with many râles in the right lower lobe and the right axilla. The borders of the heart could not be made out. There was a definite gallop rhythm, and the heart sounds were of poor quality; no murmurs were heard. The liver and spleen were palpable 2 fingerbreadths and 1 fingerbreadth below the costal margin, respectively. There were no other masses or tenderness. Roentgenograms of the chest revealed a markedly enlarged heart and fine mottling of both lung fields. The radiologic report was cardiac dilatation and pulmonary edema, consistent with circulatory collapse. The hemoglobin was 50 per cent and the white blood cell count 13,000, with 50 per cent polymorphonuclear leukocytes.

On the patient's return to the ward following roentgenologic examination of the chest, she became dyspneic, and despite artificial respiration and stimulants she died 2 hours and 5 minutes after admission. The clinical diagnosis was pneumonia with cardiac failure.

Autopsy Findings

Autopsy, performed 2 hours after death, was restricted to the thorax and abdomen. The body was well developed and well nourished.

The right and left pleural cavities contained 32 and 50 cc. of clear, yellow fluid, respectively. The liver edge extended 5 cm. below the xiphoid process and the spleen 2 cm. below the left costal margin. The heart weighed 90 gm., as compared with a normal weight for this age of 44 gm. The myocardium was pale red-brown and flabby. There were no congenital abnormalities. The weight of both lungs was 257 gm., compared with the normal weight for this age of 121 gm. Both were congested, with increased consistency and decreased crepitance. The cut surface revealed bright pink tissue with large amounts of frothy pink fluid exuding. In some areas it exhibited a meaty, red appearance. The spleen and liver were both slightly enlarged, and there was gross evidence of fatty change of the liver. No other important gross findings were in evidence. Cultures of the heart's blood and fluid from the thoracic cavity were negative, but in cultures of the right lung was grown a type 19 pneumococcus. The gross findings were pneumonitis and hypertrophy of the heart, the cause of which was obscure.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, and Foot's reticulum stains, and scharlach R. The sections of the left ventricle revealed a diffuse interstitial infiltration by lymphocytes, predominantly, but there were occasional plasma cells, polymorphonuclear leukocytes, a rare eosinophil, and large mononuclear cells with large vesicular nuclei and abundant eosinophilic cytoplasm. Although the exudation was widespread in the left ventricle, it was not uniform throughout. There were many focal collections, and in some areas these were closely packed, particularly adjacent to the pericardium, where they formed groups in which the continuity of the muscle fibers was interrupted. There was a minimal involvement of the pericardium, with foci and scattered exudative cells beneath this layer. There was no inflammatory reaction on the epicardial surface. The endocardium was involved also in that portion contiguous with the myocardium, but not to a marked degree. The endocardium of the left auricle was thickened by an increase in the fibrous tissue. It was also edematous, but the endothelium was intact and without exudation. A section of a valve leaflet revealed some separation and fragmentation of the connective tissue, but there was no evidence of thickening, vascularization, or cellular

infiltration. The cardiac musculature exhibited little definite evidence of degeneration. The myocardial fibers were well preserved and without loss of striations, and only in a few areas were there occasional muscle fibers with fragmentation and shrinking, and distorted pyknotic nuclei. There was, however, a diffuse interstitial edema. The process in the left auricle was similar to that in the left ventricle but was much less severe. There were small focal collections of lymphocytes. The right ventricle also presented the same histologic picture as the left auricle. There appeared to be no involvement of the vascular system of the myocardium; neither was there any evidence of Aschoff bodies. Foot's reticulum stain was noncontributory, nor did Gram's or Giemsa's stains demonstrate bacteria or inclusion bodies. The scharlach R stain showed no demonstrable increase in stainable fat.

The other microscopic findings were congestion and edema of the lungs, a mild interstitial pneumonia, toxic splenitis, toxic adenitis, and fatty change of the liver.

Case 3

D. C., an 11-months-old girl, entered the hospital for the second time on August 28, 1946 (no. A-301221), with cough and fever of 2 weeks' duration. The family history was noncontributory. The patient had two older siblings living and well. The mother's pregnancy had been uneventful except for edema of the ankles, and the delivery had been normal. The neonatal course was without complication until at 2½ months of age, when the patient developed an upper respiratory infection followed at 3 months by diarrhea. At this time she was first admitted to the hospital. She was placed on penicillin and chemotherapy, but in spite of this the diarrhea persisted for the first few weeks. The temperature ranged from normal to 103°F. Twenty-three days after admission, bilateral mastoiditis was demonstrated roentgenologically. Bilateral antrotomy was performed, but the patient showed little improvement until 1 week after the operation, when improvement was rapid. She was discharged home on February 3, 1946, 31 days after admission.

The patient was apparently well until 2 weeks before the second admission, when she developed a cough, fever, and grunting respirations. Four days before admission she received her last injection of whooping cough vaccine, and a mixture of diphtheria and tetanus toxoids.

The physical examination revealed an acutely ill infant with respirations of 84 and a temperature of 103°F. She became cyanotic on crying. The chest revealed slight inspiratory retraction, hyperresonance, and expiratory wheezes. The liver was palpable but there were no other abdominal masses. Fluoroscopy revealed an enlarged heart and poorly aerated lungs. Roentgenologic examination of the chest on the first admission had shown the heart to be of normal size. The white blood cell count was 15,000, with 60 per cent lymphocytes and 28 per cent polymorphonuclear leukocytes. The infant failed rapidly in spite of oxygen, steam, penicillin, sulfadiazine, and aminophylline and died 15 hours after admission. The clinical diagnosis was acute bronchiolitis.

Autopsy Findings

Autopsy, performed 6 hours after death, revealed a well developed and nourished female infant with slight pitting edema of the feet and

cyanosis of the lips. Examination was restricted to the thorax and abdomen. The liver edge was 4 cm. below the xiphoid process. There was no fluid in either pleural cavity. The heart was enlarged and dilated. The myocardium seemed normal except for the region of the interventricular septum, where there was some gray streaking. The endocardium was smooth and glistening except for moderate opacity and thickening in the region of the septum. The coronary arteries were injected by the technic of Schlesinger [4] and no anomalies or defects were found. The lungs exhibited some atelectasis and emphysema. There was evidence to support the diagnosis of aspiration pneumonia. The abdominal viscera were congested and edematous. In cultures of the heart's blood *Escherichia coli* was grown, but the lung cultures were negative. The gross findings were consistent with congestive cardiac failure. The cause for cardiac hypertrophy was not established.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Multiple sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, Foot's reticulum, and Weigert's elastic tissue stains, and scharlach R. All sections of the left ventricle were histologically similar. They presented a diffuse interstitial infiltration by lymphocytes with occasional polymorphonuclear leukocytes, plasma cells, and a rare eosinophil. The exudation was not uniform and in many areas exhibited large, ovoid collections of lymphocytes. In these particular areas the muscle fibers had lost their continuity and were not recognizable as such. In the remaining myocardium there was little evidence of damage except in one area, where there was a fine interstitial fibrosis adjacent to the endocardium. There was an occasional muscle fiber which showed hyaline degeneration, but the majority appeared histologically normal. There was, however, a diffuse, moderate, interstitial edema. The epicardium was not involved except that the exudate seemed to be more abundant in the myocardium adjacent to it. Nowhere was there evidence of pericarditis. The left ventricular endocardium was thickened by fibrous tissue, and there was a slight infiltration of this tissue that was contiguous with the myocardium. The interventricular myocardium presented an identical picture with an abundant lymphocytic exudate and edema that was more severe than that seen in the left ventricle. Sections of the right ventricle and left auricle also showed a similar interstitial exudation. Here the focal collections and scattered lymphocytes were much less abundant. Sections of the right auricle presented only a minimal inflammatory reaction. There appeared to be no abnormality of the coronary arteries. Sections of the mitral, aortic, and tricuspid valves showed no lesions except for slight edema. Weigert's stain

revealed increased elastic tissue in the left ventricular endocardium. Foot's reticulum stain showed no apparent increase in reticulum except for one section of the left ventricle, which revealed an increase in fibrous tissue of the myocardium, near the endocardium. Giemsa's and Gram's stains demonstrated no evidence of bacteria or inclusion bodies. Scharlach R stain revealed no increase in stainable fat.

The other microscopic findings were a mild interstitial pneumonia, an aspiration pneumonia, edema and congestion of the lungs, lymphoid hyperplasia, and fatty change of the liver.

Case 4

R. D., a 12-months-old boy, was admitted on October 15, 1946 (no. A-46-235). He was in good health until 2 weeks before admission, when suddenly, while sitting in a chair, he began to scream as if in pain, developed pallor, and sweated profusely. This persisted for 15 minutes and subsided as abruptly as it had begun. Following this episode the patient was irritable, ate poorly, and sat up in order to sleep. In the 2 weeks preceding admission there were three or four attacks of a similar nature, lasting the same length of time, and with some of them there was retching but no vomiting. They had no particular relationship to activity or feeding. There was no fever, but a gradual increase in dyspnea, malaise, anorexia, and some blueness of the lips were noted.

Physical examination revealed an acutely ill infant, well nourished and well developed. The temperature was 100.8°F.; pulse, 180; respirations, 78. The blood pressure was 90/65 mm. of Hg. The skin was moist and gray-white. No edema was present. There were fine inspiratory râles over both lung bases. The heart was enlarged to percussion and the sounds were of poor quality. The rate was rapid but the rhythm was regular. The liver edge was felt at the iliac crest and the spleen was just palpable. Roentgenologic examination of the chest showed the heart to be markedly enlarged to the right and left, with considerable enlargement of the pulmonary vessels and congestion and edema of the lung fields.

The electrocardiogram showed sinus tachycardia, right axis deviation, and low T waves in all leads. Examination of the urine showed 0 to 1 plus albumin. The hemoglobin ranged from 9.6 to 13.1 gm.; red blood cells, 4.0 to 4.75 millions; white blood cells, 7,100 to 16,500, with 5 to 54 per cent polymorphonuclear leukocytes, 36 to 94 per cent lymphocytes, 8 to 1 per cent monocytes, and 1 per cent eosinophils. No pathogens grew in a culture from the nose. A glucose-tolerance test and an adrenalin-tolerance test gave normal reactions.

The patient improved under treatment with oxygen, digitalization, thiocalcin by rectum, and sedation. The pulse dropped to 108 and the liver edge was palpable at the umbilicus. Repeated roentgenograms of the chest showed clearing of the lungs and a gradual decrease in size of the cardiac silhouette. The patient did well and was discharged home on the 24th hospital day, although the pulse remained above 100. On the day after discharge he developed an upper respiratory infection, followed in 24 hours by anorexia, dyspnea, and an ashen-gray color. He was readmitted on the 3rd day after discharge. The findings were similar to those of the first admission, and similar therapy was instituted with the addition of chemotherapy. However, chemotherapy was stopped within 2 days because of a decrease in the white blood cell count and a marked shift to lymphocytes. The second electrocardiogram showed sinus tachycardia, right axis deviation, low T-1, diphasic T-2, and inverted T-3. Aside from nasopharyngitis, the patient did well and was discharged to a convalescent home 3½ weeks after the second admission. Within

24 hours after discharge he developed fever, cough, dyspnea, and restlessness, and was readmitted in a moribund state. He died 2 hours after admission, approximately 66 days after the onset of illness. The clinical diagnoses were myocarditis, pneumonia, and congestive cardiac failure.

Autopsy Findings

Autopsy was performed 12 hours after death. Each pleural cavity contained 50 cc. of clear, yellow fluid, while the pericardial sac contained 20 cc. The heart was dilated and weighed 111 gm., as compared with a normal weight of 45 gm. The pericardium was smooth and glistening and presented several small petechiae. The myocardium was soft, flabby, and red-brown, and in some areas was paler than is normal. The endocardium of the left ventricle was opaque and on section the surface appeared to be slightly thickened. No congenital abnormalities were seen. The lungs were increased in weight, congested, and edematous. There was some gross evidence of pneumonitis. There was congestion of the abdominal viscera with enlargement of both the liver and the spleen. The brain was edematous and congested. The findings were consistent with congestive cardiac failure. A post-mortem blood culture yielded no growth, while from the lung alpha streptococcus and *Micrococcus catarrhalis* were grown. The cause for hypertrophy of the heart was not found.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Sections of the heart were stained with hematoxylin and eosin, Gram's, Giemsa's, Foot's reticulum, Weigert's elastic, and scharlach R stains. Sections of the left ventricle revealed an interstitial infiltration by lymphocytes, polymorphonuclear leukocytes, plasma cells, and eosinophils. The predominant cells were lymphocytic. The infiltrate was not uniform throughout the sections. In some, it was abundant with many focal collections, and in areas it appeared to be more prominent in the vicinity of the pericardium. Other sections showed minimal involvement. The moderate amount of subepicardial fat showed some cellular infiltration, but nowhere did there appear to be any recognizable pericarditis. Many of the exudative cells were in the vicinity of the coronary arteries, but there was no evidence of a vascular lesion. There was moderate thickening of the left ventricular endocardium by fibrous tissue, but here again there was minimal involvement of the endocardium by inflammatory cells in areas contiguous with the myocardium. There was moderate interstitial edema of the myocardium, but there was little evidence of degeneration. Only in the focal areas of lymphocytes were the myocardial fibers absent. In general, the cardiac muscle was well preserved and only occasionally was there a pyknotic nucleus. There was moderate con-

gestion of all blood vessels. The right ventricle presented similar interstitial infiltration, predominantly by lymphocytes. The exudate was spotty and much less severe than that of the left ventricle. Here again it was more abundant near the epicardium. Sections of the left and right auricle showed only an occasional focus of lymphocytes in close proximity to small arterioles; the Weigert stain exhibited a slight increase in elastic fibers in the left ventricular myocardium. Giemsa's and Gram's stains were negative for bacterial inclusion bodies. Foot's reticulum stain showed no increase in connective tissue. Glycogen and scharlach R stains gave only negative results. Sections of the mitral valve revealed no evidence of abnormal structure.

Other microscopic findings were a terminal bronchopneumonia, moderate congestion of liver and spleen, and toxic splenitis.

Discussion

It is not the primary purpose of this paper to discuss the clinical aspects of these cases, but there were striking similarities in all four. The onset of symptoms was relatively of short duration, ranging from 3 hours to 3 weeks. Except for case 3, the mothers had observed cyanosis, dyspnea, and anorexia. The family and past histories were all essentially noncontributory except for case 3, and these factors will be discussed in relation to possible etiologic agents. On admission to the hospital the patients were acutely ill, with rapid pulse, labored respirations, and an elevated temperature. There was enlargement of the heart either by physical examination or roentgenologically. Several electrocardiograms were done in case 4 and all showed sinus tachycardia, right axis deviation, and low T-waves in all leads, which suggested myocarditis or a digitalis effect. The lungs presented positive findings by percussion and auscultation, except for case 1. The liver and spleen were palpable in all cases except case 3, in which only the liver was felt. The course in the hospital was of short duration in the first 3 cases, and was measured in hours. Only in case 4 did the patient survive sufficiently long for an adequate clinical study. The pathologic findings were essentially the same. The bodies were well developed and well nourished. The heart was enlarged and dilated, and an adequate cause for hypertrophy was not demonstrated at the time of the autopsy. Congenital abnormalities were lacking. The coronary circulation followed the usual anatomic pattern, although in case 1 the prosector believed that he was dealing with a possible coronary occlusion. Microscopic sections of the coronary arteries, however, did not substantiate the gross impression. None of the types of coronary occlusion described by Stryker [5] were found. The myocardial lesions

of case 1 did not resemble either tuberculosis or syphilis. Neither did this case resemble the characteristic granulomatous lesion described by Saphir,[2] Magner,[6] and Jonas.[7] This form of myocarditis is a clearly defined one, not unlike known granulomas and sometimes confused with syphilis and tuberculosis. It may well be that the heart of case 1 is an example of a myocarditis developing in utero. The remaining cases were all histologically similar and varied only in the amount of exudate. In summarizing the microscopic picture, the myocardium was diffusely involved by an interstitial exudate, consisting primarily of lymphocytes, but with an occasional polymorphonuclear leukocyte, plasma cell, eosinophil, and large mononuclear cell. There was moderate to severe interstitial edema, but there was little evidence of myocardial damage. Nowhere was there a specific lesion found, such as the Aschoff body. The endocardium and epicardium showed only minimal involvement. In cases 3 and 4 there was endocardial fibrosis, which was interpreted as fibro-elastosis (fetal endomyocarditis). The ventricular endocardium was moderately thickened by elastic fibers with extension into the underlying myocardium. In only one case (no. 3) was there patchy fibrosis of the myocardium. The lungs of all cases showed gross and microscopic evidence of interstitial pneumonia or bronchopneumonia. This was a terminal complication and histologically the inflammatory lesions of the heart antedated the pulmonary process. It is important to point out that no other major pathologic lesion was found. The incidental findings, such as the congested abdominal viscera, could all be explained on the basis of congestive cardiac failure. These 3 cases (2, 3, and 4), from the clinical and necropsy observations, appear to fall into the second more common type of diffuse interstitial myocarditis.

The etiologic basis for myocarditis of this type is unknown. The multiplicity of agents suggested in the literature makes this evident. For the granulomatous type it has been stated that tuberculosis or syphilis may be a possible cause even though the causative organisms are not demonstrated. Baker and Brian[8] have called attention to blastomycetes as producing granulomas in the heart, and it appears possible that other organisms could produce this same type. Lillie and Francis[9] have shown that *Pasteurella tularensis* can produce granulomas in the hearts of experimental animals.

The diffuse form of interstitial myocarditis has appeared more frequently in the literature, and as a result more agents have been suggested as possible factors in causing this pathologic process. Severe burns, upper respiratory infections, toxins, influenza, and infectious diseases have been mentioned. According to other case reports, the

administration of adrenalin has produced myocarditis of a similar type. Experimentally, it has been produced with sparteine and adrenalin combined. Bismuth and the arsenicals have been attributed as factors in producing this condition. In relation to this possible allergic response, French and Weller [10] reported 126 cases of interstitial myocarditis in which sulfonamide therapy was thought to be the common etiologic agent. Wells and Sax [11] recently have reported a case in which they believe sulfadiazine might well have been the cause of a myocarditis. Vitamin deficiencies, idiosyncrasies to sulfur ointment, and alcohol have been mentioned. Helwig and Schmidt,[12] in 1945, reported the death of two chimpanzees in their laboratory from cardiac failure and pulmonary edema. Histologically, the principal finding was an acute interstitial myocarditis. Pleural fluid from one animal was passed through 122 mice and with rare exceptions produced paralysis and acute myelitis. This same material has been used to produce similar myocarditis in guinea-pigs and rabbits. Finland, Parker, Barnes, and Joliffe [13] have reported two cases of influenza A pneumonia with a nonbacterial myocarditis. This would suggest that a virus infection may be linked with myocarditis of this type.

In reviewing the possible etiologic factors reported in the literature, it would seem that the majority of them could be eliminated in the 4 cases reported here, particularly in this age group.

In case 1, the cardiac lesion of this 21-day-old infant was nonspecific, although it suggested more of a granulomatous process. Histologically, it did not resemble a gumma or tuberculosis. Levaditi's stain and acid-fast stains were both negative. Serologic examination of both parents was negative. Another possible factor which must be considered is a coronary occlusion, even though a careful examination of the heart was made grossly and microscopically. At this relatively early age an infectious process developing in utero must be thought of also.

In case 2 there was the history of the mother developing pneumonia on the fourth day post-partum, and for that reason the baby was removed from breast feedings. This infant also contracted an upper respiratory infection 5 weeks before the onset of the terminal illness.

In case 3 there was an upper respiratory infection at 3 months of age followed by bilateral otitis media. At this time chemotherapy was used and it was also administered during the brief last illness. Four days before the last admission she received the last injection of whooping cough vaccine, tetanus and diphtheria toxoid. On admission to the hospital a diagnosis of an upper respiratory infection was made. One might possibly conceive that the myocarditis in these two cases (2 and 3) was produced on an allergic basis since both gave a history of recent and

former upper respiratory tract infections. Mallory and Keefer [14] have observed focal accumulations of lymphocytes, plasma cells, and histiocytes beneath the endocardium, and to a lesser extent in the myocardium in cases of hemolytic streptococcus infections. Similar lesions were found in the kidney, liver, lungs, spleen, and pancreas in their cases. They suggested that these lesions were due in part to an antigen-antibody reaction. Although the lesions which they demonstrated in the heart were focal, it seems possible that a diffuse interstitial myocarditis could develop on the same basis.

"Sulfa drug" sensitivity as a possible etiologic factor appears remote. There was a history of chemotherapy at 3 months of age, and as far as it is known there was no further history of the drug until the terminal illness. Since the infant died 15 hours after admission, it would seem unlikely that the amount of myocardial exudation observed could possibly have occurred in that brief time. It is also evident that the patient had cardiac involvement at the time of admission.

The rôle of the inoculation in case 3 cannot be evaluated except that it might possibly have been an etiologic factor.

Although case 4 was the only one in this series in which the patient lived long enough for an adequate clinical study, there is no lead to a possible etiologic factor.

The pulmonary lesions in all four cases were essentially similar. Histologically, they did not represent severe inflammatory processes, but rather a terminal interstitial pneumonia. It is also interesting to note that all blood cultures were negative, although pathogenic organisms were cultured from the lungs.

Summary

In four instances of interstitial myocarditis in children, three were of the diffuse interstitial type and one could be included in the granulomatous group.

At present there is no known cause for myocarditis of this type.

The main purpose of this paper is to direct renewed interest toward myocarditis of an interesting and poorly understood type, which causes death in infancy as well as in later life.

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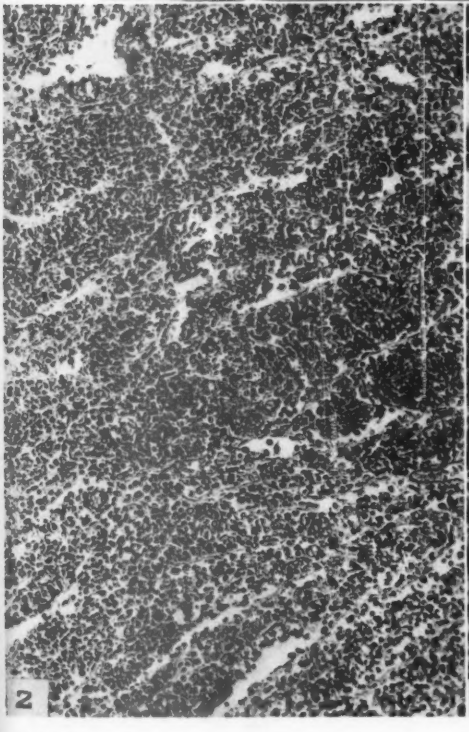
DESCRIPTION OF PLATES

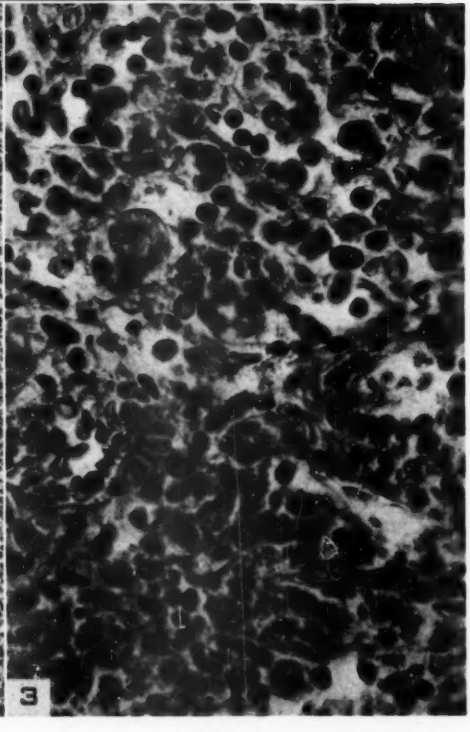
PLATE 202

FIG. 1. Case 2. Photograph of organs *in situ*. Of note is the relative size of the heart.

FIG. 2. Case 1. Low-power view of the left ventricle in the area of greatest damage, showing leukocytic infiltration, abundant capillaries, and loss of myocardial fibers. Hematoxylin and eosin stain. × 140.

FIG. 3. Case 1. Higher power view of the same area seen in Figure 2. Hematoxylin and eosin stain. × 500.





House

Diffuse Interstitial Myocarditis

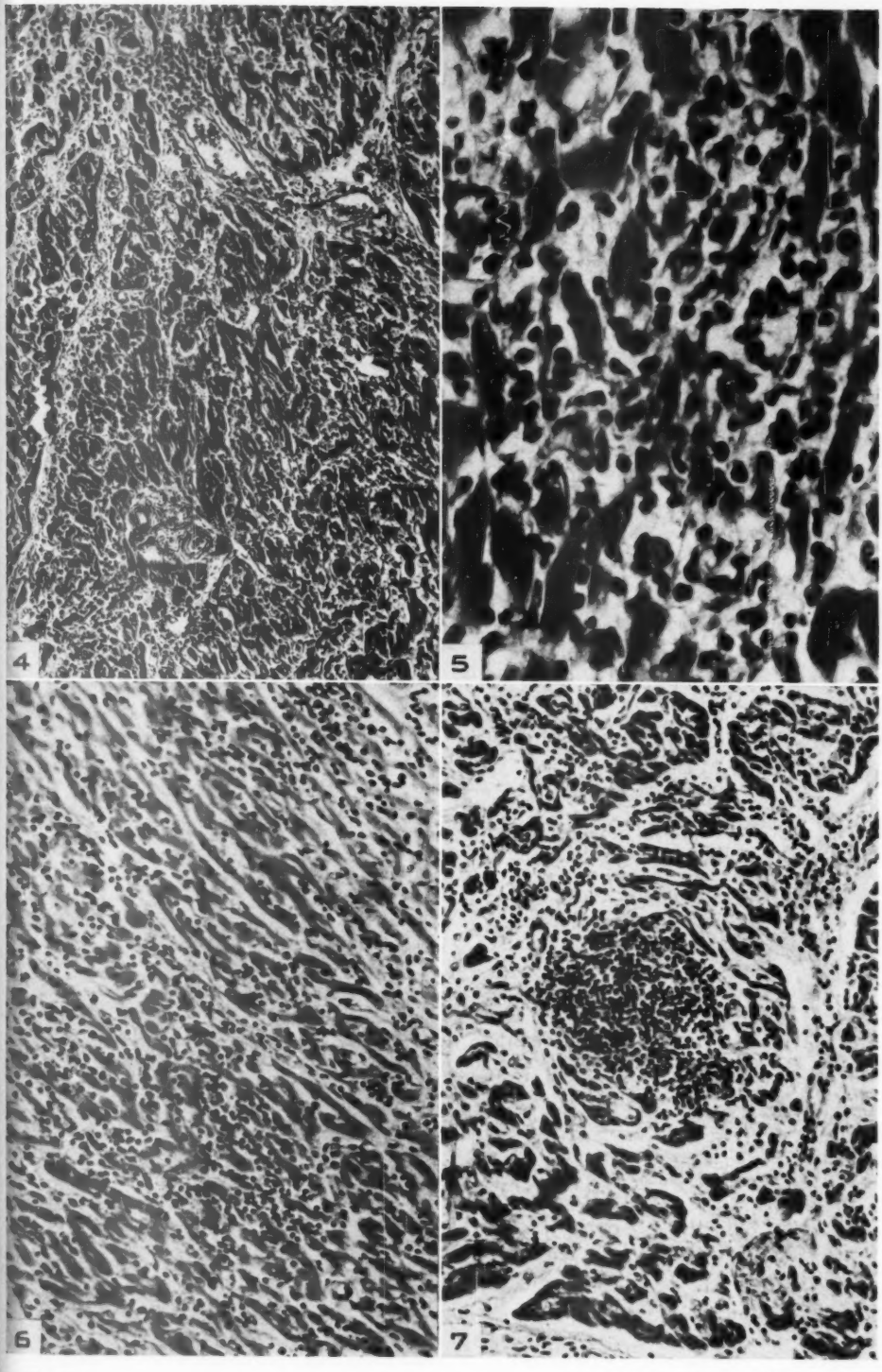
PLATE 203

FIG. 4. Case 2. A representative section of the left ventricle, showing the leukocytic infiltration. Hematoxylin and eosin stain. × 100.

FIG. 5. Case 2. A higher power view of the same area seen in Figure 4, showing loss of myocardial fibers. Hematoxylin and eosin stain. × 500.

FIG. 6. Case 3. Low-power view displaying a diffuse interstitial exudate. Hematoxylin and eosin stain. × 180.

FIG. 7. Case 3. Low-power view of another area, showing a focal collection of lymphocytes. Hematoxylin and eosin stain. × 180.



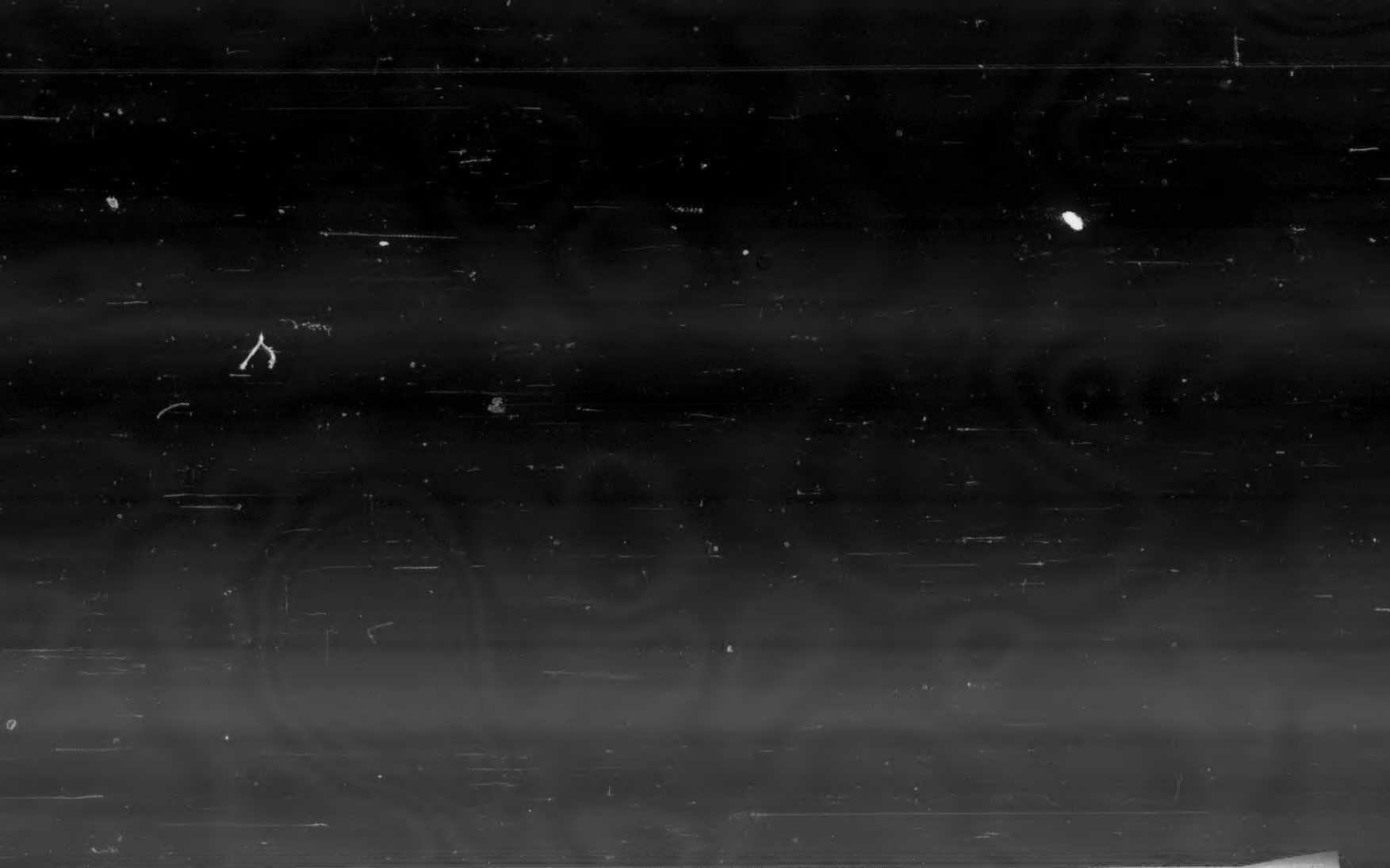
House Diffuse Interstitial Myocarditis

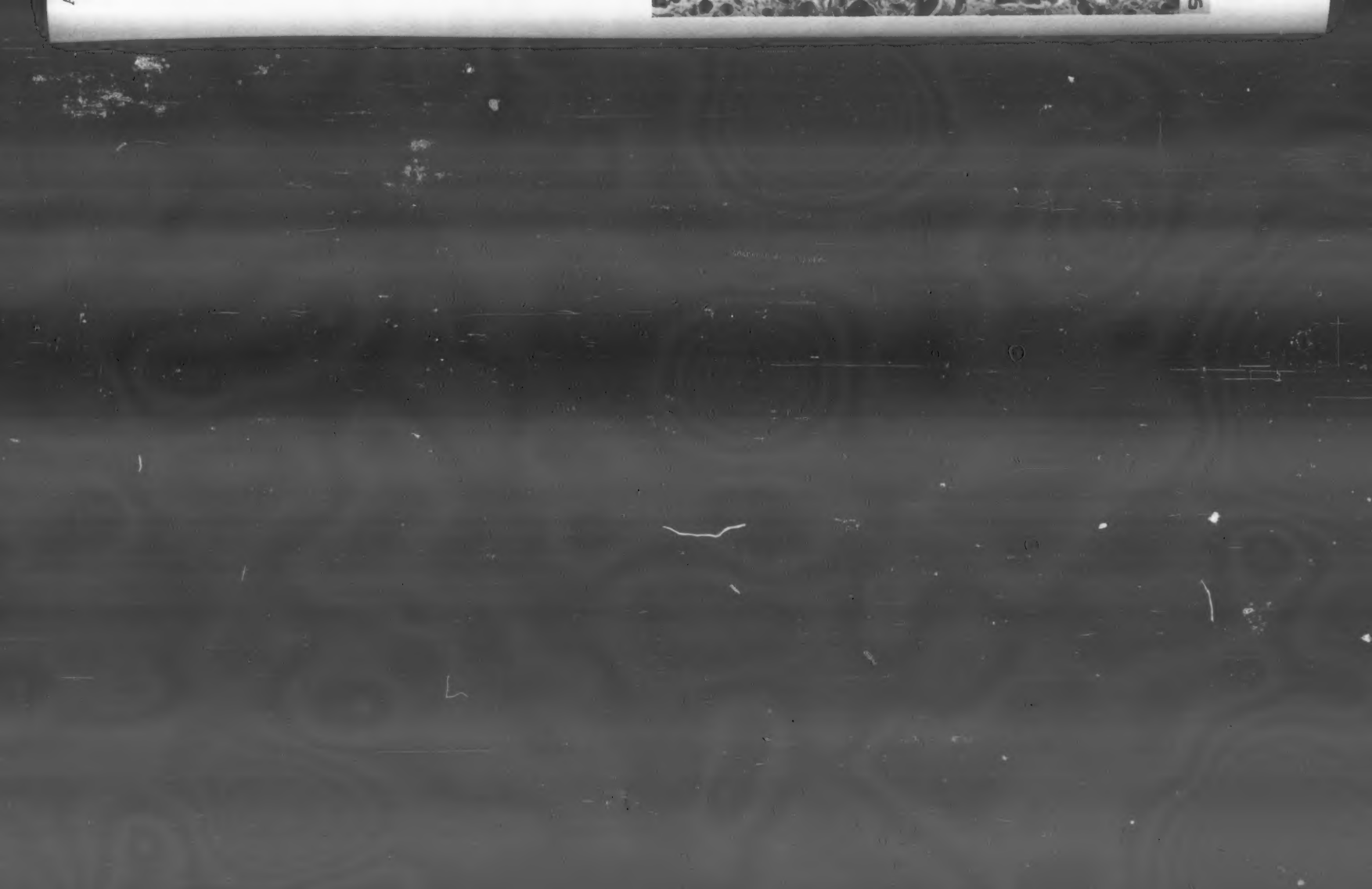
PLATE 204

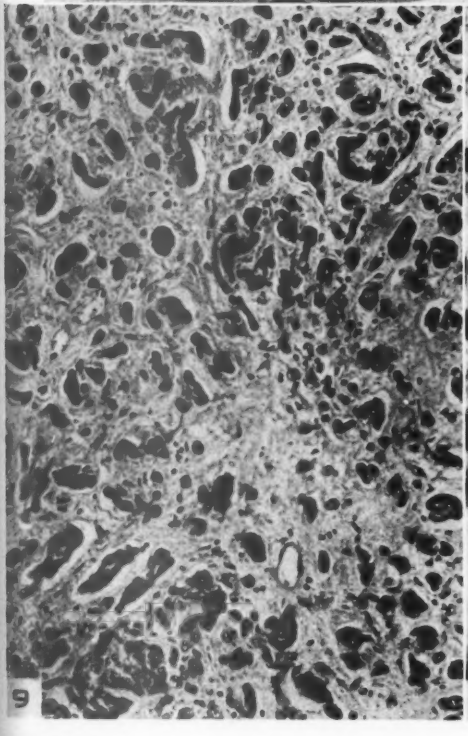
FIG. 8. Case 3. Photograph of organs *in situ*. Of note is the relative size of the heart, similar to that of Figure 1.

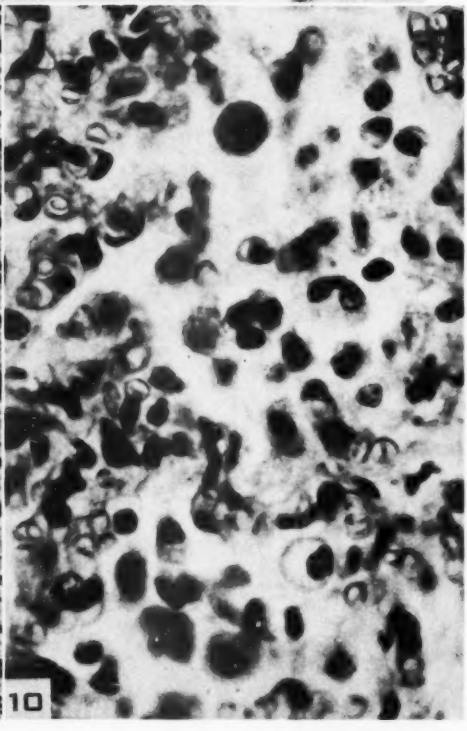
FIG. 9. Case 3. Low-power view of an area of fibrosis with minimal exudation. Hematoxylin and eosin stain. × 250.

FIG. 10. Case 3. A higher power view of the same area seen in Figure 9, showing types of exudative cells—lymphocytes, plasma cells, macrophages, and polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 500.







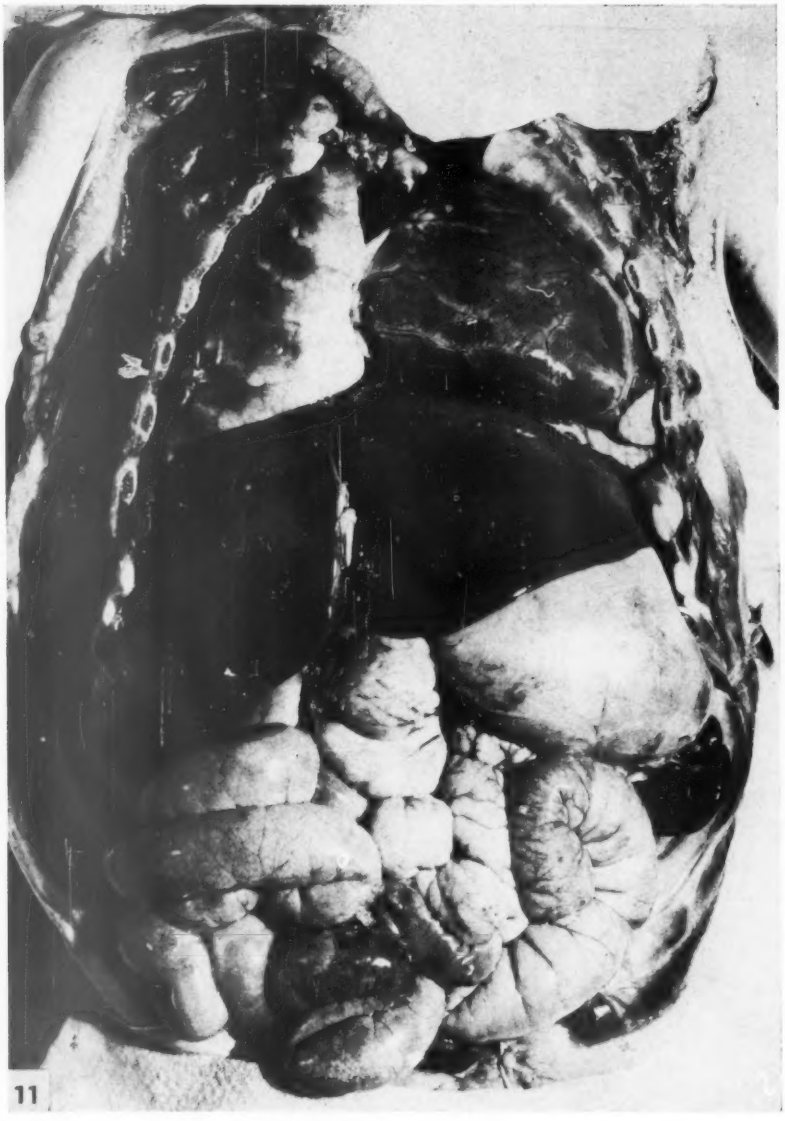


House

Diffuse Interstitial Myocarditis

PLATE 205

FIG. 11. Case 4. Photograph of organs *in situ*. Similarity to Figures 1 and 8 may be noted.



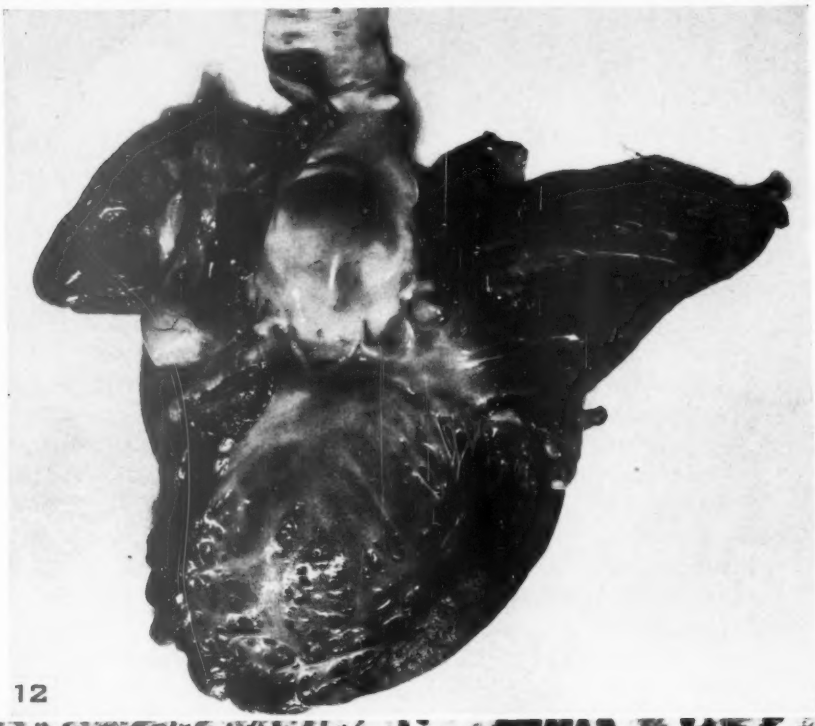
House Diffuse Interstitial Myocarditis

PLATE 206

FIG. 12. Case 4. View of the open left ventricle. There is a generalized opacity of the endocardium (fibro-elastosis).

FIG. 13. Case 4. Low-power view showing the diffuse interstitial leukocytic infiltration. Hematoxylin and eosin stain. × 140.

FIG. 14. Case 4. Higher power view of the same area seen in Figure 13. The exudate is predominantly lymphocytic in this field. Hematoxylin and eosin stain. × 500.



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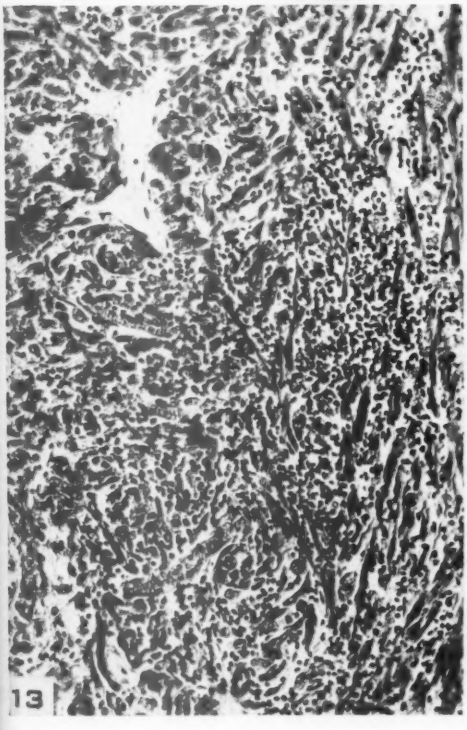

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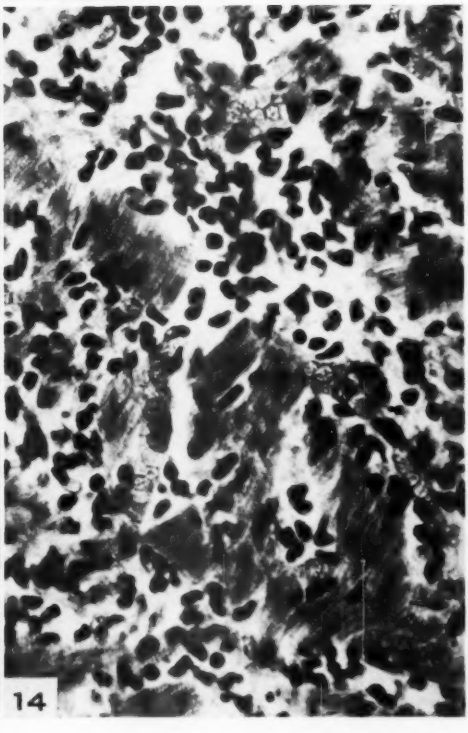

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House

Diffuse Interstitial Myocarditis

STRUCTURE OF THE GLOMERULUS OF THE HUMAN KIDNEY*

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Present opinion, following the studies of McGregor,[1] Bell,[2] and others, considers the glomerulus of the human kidney as a simple contortion of capillaries, lacking any stromal elements. In direct opposition to this, Zimmermann[3] gave a description of the mesangium—a connective tissue framework for the glomerulus. Kimmelstiel and Wilson[4] and Goormaghtigh[5-7] utilized the concept of mesangium in discussions of glomerular alterations in disease. Difference of opinion on a matter so fundamental as glomerular structure suggested a review of the matter with the periodic acid-Schiff's reagent (PAS) technic.[8,9]

This paper describes and illustrates the results of a study of the human glomerulus with particular reference to the basement membrane and the stromal elements or mesangium. The method of study is the coloring of paraffin sections by the PAS technic with which there is a selective coloring of the basement membrane of the renal tubule and glomerulus.[8,9] The report includes the description of the glomerulus in kidney disease of several types.

MATERIALS AND METHODS

Much of the material came from the autopsy and surgical series of the Jefferson-Hillman Hospital. Slides from the files of the old Hillman Hospital (1921–1944 inclusive) were colored by PAS after removing the coverslips in xylene and decolorizing in 1 per cent hydrochloric acid in 70 per cent alcohol. Cases of traumatic uremia and some normal material had been collected during the European campaign of World War II. Other specimens were sent in by various pathologists.

The time or type of fixation is not crucial in the study of the glomerular basement membrane with the PAS technic. The basement membrane is preserved in tissues in which autolysis is advanced. Zenker's formol is the best of the routine fixatives for the glomerular basement membrane and Bouin's fluid and Holmgren's[9a] 4 per cent sub-acetate of lead are useful because of their ability to preserve carbohydrate in sections. Dehydration by autotechnicon† preserves somewhat less of the PAS-colored material in the glomerular basement membrane than

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† The Technicon Co., 215 E. 149th St., New York 51, N.Y.

the slower technics. The staining technic for the basement membrane is that previously described in detail.[9]

Where blocks were available, sections were cut generally at 6 μ. Thin sections at 3 μ and thick sections at 25 μ were cut on occasion. A few serial sets were made.

The Normal Glomerular Basement Membrane

In thick sections, the glomerular loops were seen as a tangled skein of tubes of inconstant caliber. Unequal dilatation of the loops occurred after death from heart failure and the larger loops could be traced to the efferent vessel. All of the loops were covered with a deeply colored basement membrane which showed apparent points of concentration where loops lay in contact.

In 3 μ sections, it could be seen that the afferent vessel divided into a number of branches, each having a basement membrane continuous with that of the arteriole. If the loop was situated peripherally near the root of the tuft, its basement membrane connected with that of the parietal layer of Bowman's capsule. A short distance after the division into branches, *i.e.*, the formation of loops, or at times immediately, the loops became contorted and could not be followed adequately.

In sections 6 to 8 μ thick, a loop could be seen at times to curve about a central axis (Fig. 1). The basement membrane covered the loops and an intervening axial space. Studying this same area in the thinnest sections, it could be seen that there might be a nucleus in the central (axial) portion or, more commonly, the space might appear empty except, perhaps, for cytoplasm (Fig. 2). Frequently the axial cells projected knobs of their cytoplasm into two or more adjacent loops. In some cases the portion of the basement membrane which lay over the axial cells, *i.e.*, between the loops, could be seen to be thickened (Fig. 2), producing one variety of the "axial thickening" of Kimmelstiel.[4]

It was impossible to define the cells in the axial space as endothelium or as epithelium. Their nuclei stained like endothelial cell nuclei with phosphotungstic acid hematoxylin. Calling them "third type" cells might serve for purposes of description while prescinding from the question of their origin. No myofibrils were seen in their cytoplasm, either in material taken for biopsy or from autopsies performed from 30 to 45 minutes after death, after any fixative, or with any stain.

Sometimes, as McGregor [1] pointed out, a coarse axial fiber in the glomerulus could be traced to the parietal basement membrane of Bowman's capsule, this being especially noticeable when the latter was thickened (Fig. 3). That represented the only connection that could

be recognized between the axial space and an extraglomerular structure.

In summary, the glomerular basement membrane in the normal human kidney is formed from the basement membrane of Bowman's capsule which becomes attached to the arterioles of the glomerular root. Covering the tuft, the glomerular basement membrane encloses: (1) the capillaries of the loops which have no basement membrane of their own; (2) the endothelial cells of the capillaries; (3) the axial space between the loops where the basement membrane is reflected over the loops; (4) the infrequent axial or third type cell in these spaces; and (5) occasional fibers which pass into the glomerulus from Bowman's capsule.

It is possible that only epithelial basement membrane is being shown in the glomerulus with PAS. Capillary linings are shown elsewhere. The studies of abnormal glomeruli to be described make this improbable.

The Glomerulus in Some Diseases

The glomeruli in renal diseases of specific types have been studied because of the apparent involvement of the axial space or of the description of mesangial lesions. The series includes:

Diabetes mellitus	4 cases
Intercapillary glomerulosclerosis	6 cases (2 nondiabetic)
Acute glomerulonephritis	10 cases
Eclampsia	16 cases
Pregnancy controls	12 cases
Wounding (crush) kidneys	10 cases*

Thin microscopic sections of kidneys in diabetes mellitus, colored with PAS, were the best material in which to recognize the axial space. The axial area always was prominent and colored strongly with PAS. It usually appeared finely fibrillar, as Bell [2] described, although Bell considered the fibrils to be intracapillary. These fibrils were colored by PAS while the fibrils of muscle cells were not. The intercapillary glomerulosclerosis of Kimmelstiel and Wilson,[4] when it occurs in diabetes, appears to be an accumulation of a PAS-positive material in the axial space and need not be accompanied by thickening of the basement membrane of the glomerulus.

The kidneys with intercapillary glomerulosclerosis showed, in the

* These 10 cases were part of a group of 35 battle casualties collected in a study of the kidney after severe wounds. The 10 were selected for review in this article because of early autopsy and adequate fixation.

earliest stages, the deposit of a strongly colored hyaline material in the axial space. The later stages showed gradual increase of the intercapillary hyaline material and usually all or many stages could be seen in one kidney. Finally, the loops were situated peripherally about the hyaline mass. The largest or most involved loops had an inner limit or "basement membrane" made up of the hyaline mass. The peripheral true basement membrane might not be thickened or distorted (Fig. 4), although this happened frequently in the non-diabetic cases. Sometimes the hyaline mass included nuclei as though this were some storage process associated with an increase of cells of the third type.

The cases of acute glomerulonephritis showed the customary increased glomerular cellularity. A proportion of this appeared to be due to endothelial proliferation. A striking feature of the situation of some of the additional cells, including polymorphonuclear leukocytes, was their localization in the axial space of the glomerulus (Fig. 5). Pyknosis of nuclei might be seen, possibly those of third type cells.

The typical glomerulus in eclampsia in thin sections appeared lobulated, the individual capillary loops being separate and discrete. This lobulation was in contrast to the lobation of acute glomerulonephritis in which the glomerulus appeared divided into two, three, or four main portions or lobes.

The capillary loops were very prominent and were covered with numerous epithelial cells. I am not sure that there had been an actual increase in the epithelial cells. It may be that autolysis had not occurred and that the normal number of cells remained. The loops themselves had the appearance of being turgid or erectile. There was a constant vacuolation of the axial space and this sometimes involved the endothelial cells (Figs. 6 and 7). There was no thickening of the basement membrane or proliferation of the endothelial cells, and there was no cellular infiltration of the mesangium. The control pregnancy material showed no similar reticulation.

The vacuolation of the axial space which produced reticulation of the glomerulus in eclampsia was a striking and characteristic lesion. It was not due to fatty change or infiltration in 2 cases available for frozen sectioning. Two cases which were clinical lipoid nephrosis—actually early chronic glomerulonephritis—showed reticulation of the axial space besides adhesions and thickening of the basement membrane. In these cases reticulation was due to triglyceride fat, colorable with sudan IV, as Bell [10] mentioned in his study of lipoid nephrosis.

I have found an occasional glomerulus in some cases of hyperten-

sion which showed reticulation, not due to lipoid. The lesion in these cases is restricted to a few glomeruli and is not a diffuse finding as in eclampsia.

Discussion

The arrangement of the basement membrane in the normal glomerulus, as demonstrated by PAS, corresponds more closely to the description of Zimmermann [3] than to that of McGregor.[1] The latter recognized the thickened portions of the glomerular basement membrane and the localization of a third type cell at these points, but considered these thickenings as "orifices into" or "passageways between" loops. The possibility of the third type cell being a "connective tissue cell" was mentioned by McGregor. Zimmermann identified the third cell as a fibrocyte, presumably a connective tissue cell.

The glomerulus is best visualized as a set of discrete, non-anastomosing, vascular channels invaginating the basement membrane of the Bowman's capsule. Rare pericapillary cells exist in the intercapillary or axial space, where the basement membrane is reflected over adjacent loops. No evidence of a double basement membrane is found, except in injected kidneys. The lesions for which MacCallum,[11] Goormaghtigh,[5-7] and others supposed a double basement membrane can be explained by the arrangement described.

Goormaghtigh considered the third cell as a muscle cell controlling the "tonus" of the glomerulus.[5-7] He described multiplication of the myocytes of the mesangium in acute glomerulonephritis and hypertrophy and hyperplasia of these cells in eclampsia. The changes in both these conditions were said to be similar to the changes in the kidney in "traumatic uremia" or the "crush" kidney (Fig. 1). I have not found the changes described by Goormaghtigh. The glomerulus in traumatic uremia is little if at all involved and shows no resemblance to the glomeruli in either acute glomerulonephritis or eclampsia.

Acute glomerulonephritis is an acute inflammation of the glomerulus which involves the capillaries and the axial space. The localization of inflammatory cells in this space and subsequent fibrosis would explain the "intercapillary" lesions (Fig. 8) of MacCallum.[11] The proliferating endothelial cells within the capillaries in acute glomerulonephritis become outlined with PAS-positive material. This "arteriole-like" appearance of the loop may furnish the basis for later intracapillary fibrosis (Fig. 9). Both intercapillary and intracapillary lesions may be seen in one glomerulus (Fig. 10).

The specific lesion of the glomerulus in eclampsia is restricted to the cells of the mesangium or axial space. These cells become swollen

and vacuolated. The resulting "reticulation" of the glomerulus in eclampsia was described [12] and illustrated [13] by Dunn. Jaffe [14] called the glomerular lesion in eclampsia an "edema of the tuft," which would agree with the present description.

Intercapillary glomerulosclerosis could be the result of "trapping" a glycoprotein in the axial space. Practically identical findings were seen in 2 cases of chronic glomerulonephritis (probably type II of Ellis [15]) and one wonders whether a chemical or serologic similarity could be found between the urinary protein in the diabetic and glomerulonephritic cases which show similar glomerular lesions. The fat in the axial space in lipoid nephrosis might be accumulated in a similar fashion or might be the result of an injury to the mesangial cells.

Allen [16] considered and dismissed the possibility of a mesangial origin for the hyaline material in intercapillary glomerulosclerosis. Reticulin stains show a lamination of the hyaline deposit in the glomerulus which Allen interpreted as evidence that the material is formed by duplication of the internal capillary wall. I do not think this is the mode of development of the lesion. In the first instance, the internal or axial capillary wall does not color with PAS (Fig. 2). On occasion, the hyalin of the intercapillary lesion can be seen to inject, as it were, one leaf of the axial space (Fig. 11). Furthermore, I do not see how it is possible in sections to differentiate fibrils from irregularly deposited hyaline material, laid down in strata.

The questions of nature and function of basement membrane are raised in pointed fashion by these studies on the glomerulus. In its original morphologic use, the term refers to hyaline linear structures visible microscopically at epithelial-connective-tissue and epithelial-capillary junctions. Empirical methods of staining have produced no definite evidence for the generally held belief that basement membrane is related to intercellular substance, known to contain the carbohydrates hyaluronic acid and chondroitin-sulfuric acid.

The glomerular basement membrane fits the criterion of position at an epithelial-capillary junction. The strong coloring which it shows with Schiff's reagent after periodic acid suggests a carbohydrate constituent.

Summary and Conclusions

The basement membrane in the normal glomerulus as shown with the PAS technic derives from Bowman's capsule and attaches to the arterioles at the glomerular root. It encloses capillary loops and certain infrequent stroma cells of the mesangium, the latter in the intercapillary or axial space.

The axial space is prominent in diabetes mellitus. It appears finely fibrillar, as was described by Bell.

Marked involvement of the axial space can be seen in various disease conditions. These can be tabulated as follows: Acute inflammation in acute glomerulonephritis; glycoprotein accumulation in intercapillary glomerulosclerosis; vacuolation and reticulation in eclampsia; lipoid accumulation in "lipoid nephrosis."

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 207

FIG. 1. Glomerulus from "crush" kidney after severe wounding. Loops at left center are arranged about a central axis. Periodic acid-Schiff's reagent (PAS). × about 300.

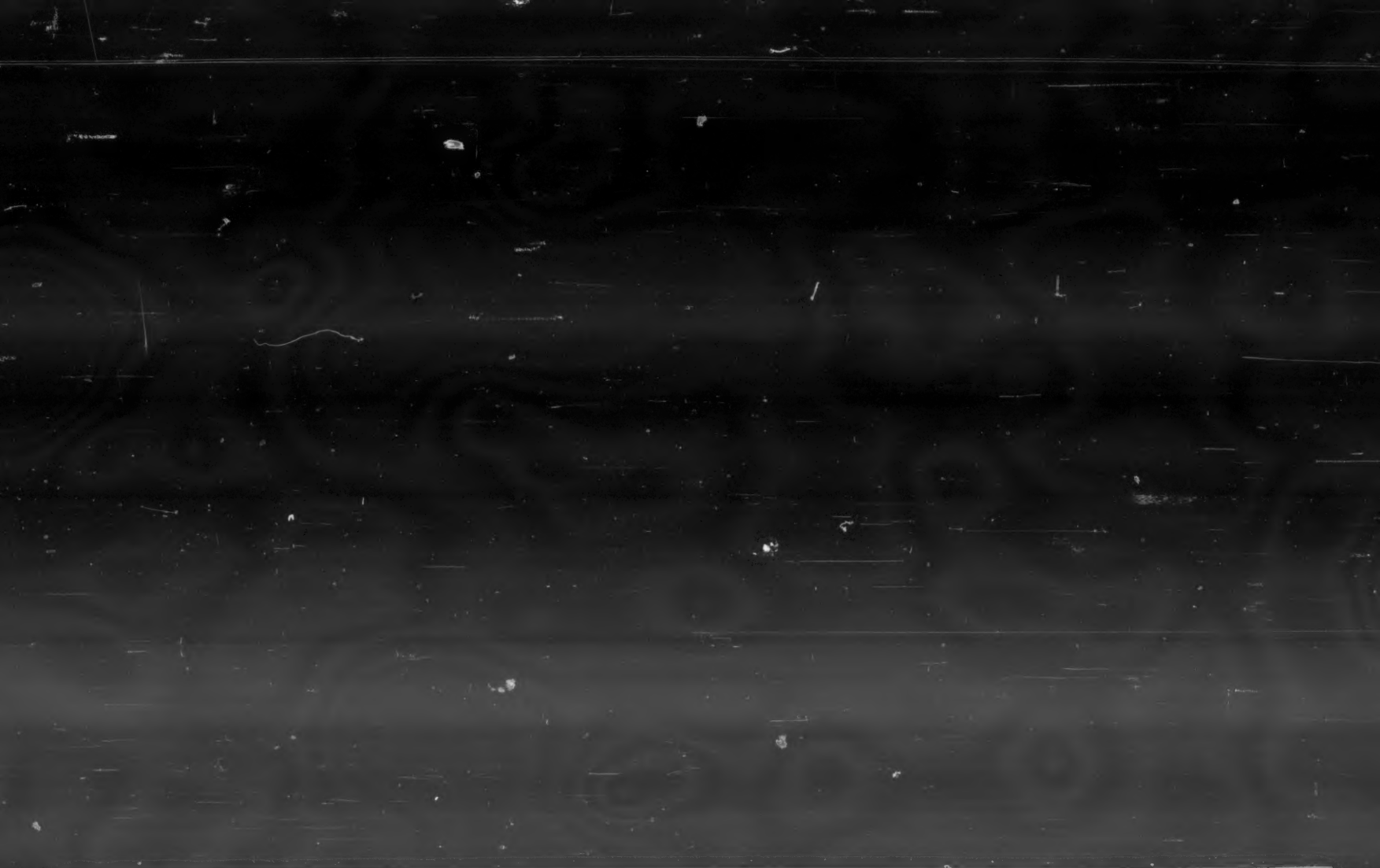
FIG. 2. Glomerular detail. The patient was a healthy adult male in shock from a chest wound 18 hours before death. Axial space is left as a T-shape by reflection of basement membrane over adjacent loops. PAS. × about 800.

FIG. 3. Same case, same glomerulus and technic as shown in Figure 2. Coarse fiber enters from the parietal basement membrane on the right. PAS. × about 300.

FIG. 4. A-657. Intercapillary glomerulosclerosis in arteriolonephrosclerosis. Centrally placed, hyaline, lamellar material with intact peripheral basement membrane. PAS, hematoxylin counterstain. × about 800.

FIG. 5. Acute glomerulonephritis. Proliferation of endothelial cells. Infiltration of axial space. PAS, hematoxylin. × about 800.

FIG. 6. Eclampsia. Vacuolation of axial space extending to endothelium. PAS, hematoxylin. × about 800.



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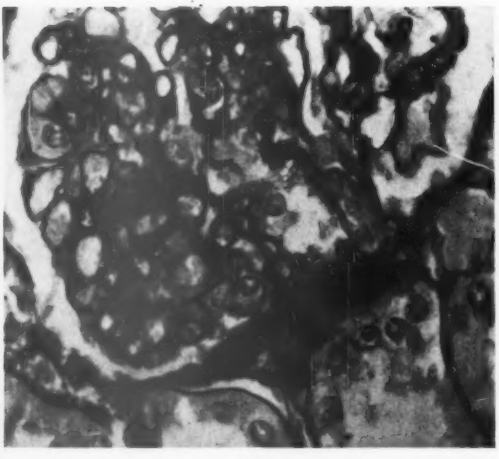

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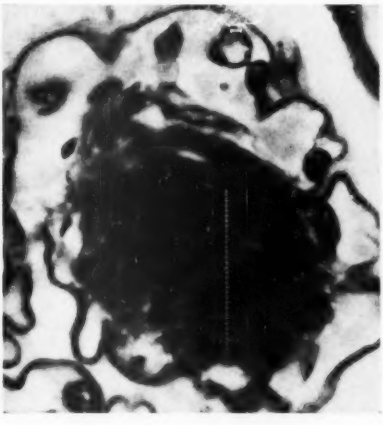

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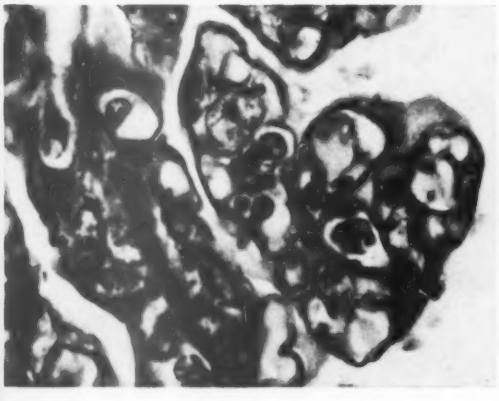

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McManus Structure of the Glomerulus

PLATE 208

FIG. 7. Eclampsia. Separate capillary loops with fine reticulation of axial space. PAS, hematoxylin. × about 300.

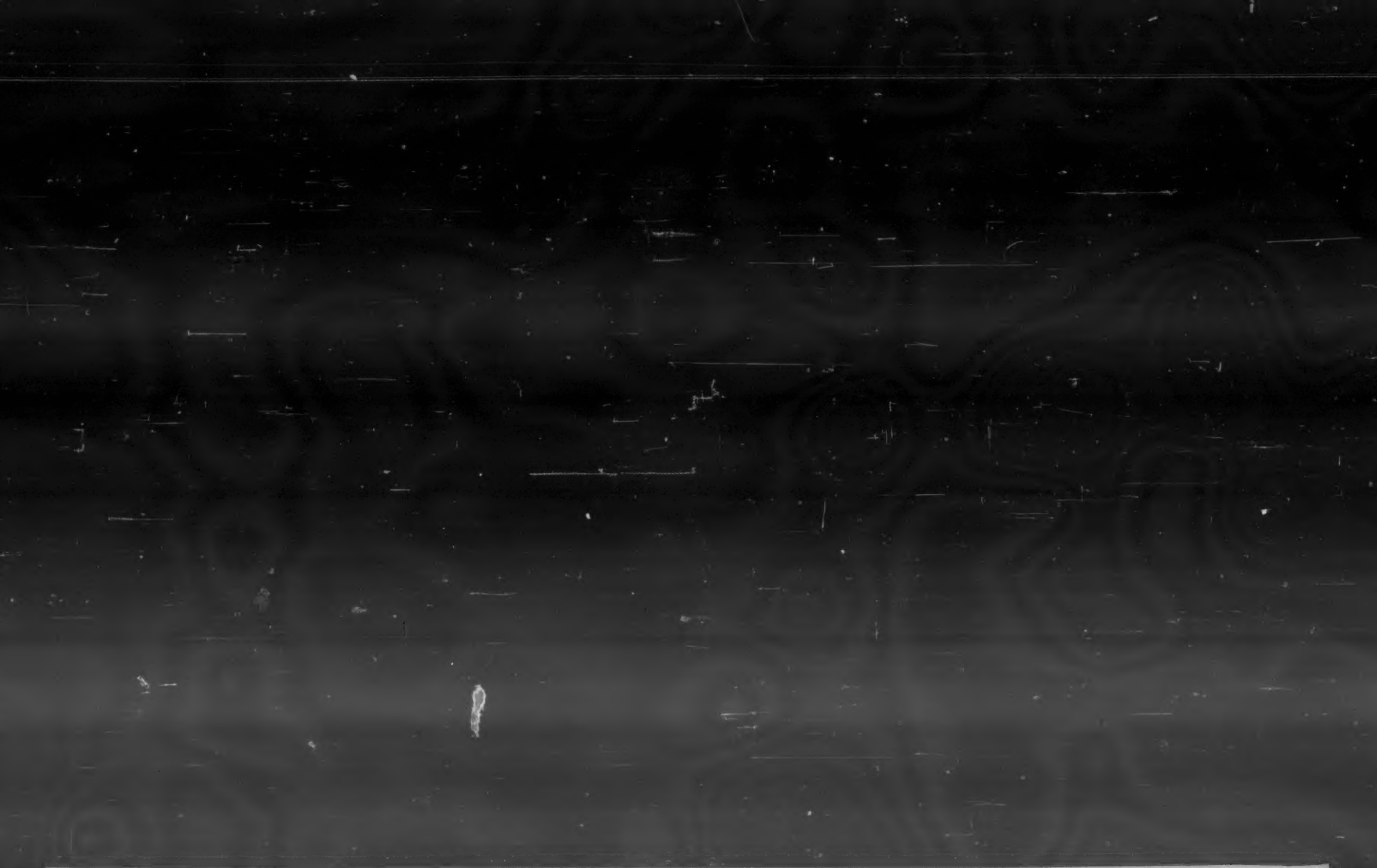
FIG. 8. Subacute glomerulonephritis. Early fibrosis of axial space with intact peripheral basement membrane in capillary loops. PAS. × about 250.

FIG. 9. Same case as shown in Figure 8. Diffuse intracapillary and axial fibrosis in progress. PAS. × about 250.

FIG. 10. Subacute glomerulonephritis. On the right of the glomerulus the peripheral loops are spared, producing intercapillary disease. To the left, the loops are involved diffusely, producing intracapillary disease. PAS. × about 250.

FIG. 11. Intercapillary glomerulosclerosis in a hypertensive diabetic patient. Of note is the peripheral displacement of loops with little change in caliber. PAS. × about 250.

FIG. 12. Higher magnification of Figure 2. × about 1250.



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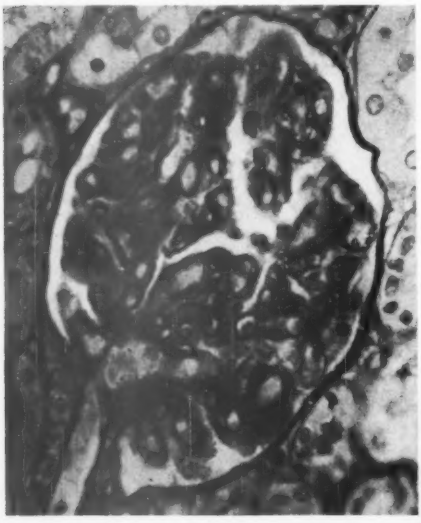

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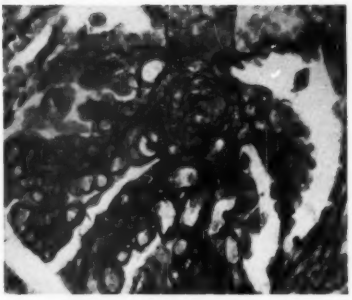

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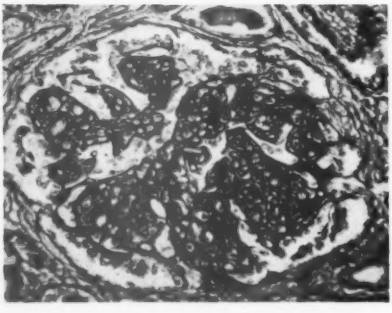

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CELLULAR CHANGES IN RABBITS DURING ANTIBODY FORMATION

I. Response to Eberthella typhosa *

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Pathologists are well aware that certain cell groups, constituting a granuloma, represent a reaction of tissues to antigen of a specific type. In most instances this tissue reaction is successful in retarding the growth of living agents or of eliminating the antigen, and this we attribute to an immune response of cells. However, while we are keenly aware of cell reactions representing an immune response, we are almost entirely unaware of cell reactions preceding immunity when antibodies are developing.

In the past 4 decades many attempts have been made to locate the site of antibody formation so that the pathologist might identify clearly the early stages of a disease process, but at present confusion still exists. Today a survey shows that there are four theories. One contends that cells of the reticulo-endothelial system phagocytize the antigen and produce the antibody. This theory is supported by "blockade" experiments of the reticulo-endothelial system,[1-8] by dye-tagged proteins,[9] and by the removal of organs.[10-12] Another theory supports the lymphatic cell as the locus of antibody formation on the basis of serologic studies showing slightly more agglutinin in lymph nodes than in serum.[13-19] A third opinion recognizes that aggregates of plasma cells contain much more antibody than other tissues.[20,21] A fourth investigator has submitted evidence that cells are not necessary for antibody formation.[22]

Therefore, under conditions of inflammation, the pathologist is not sure what interpretations are suitable when he observes unusual concentrations of reticulo-endothelial cells, lymphocytes, or plasma cells. It is toward a solution of this problem that the present report is directed.

Methods and Control Studies

All experiments herein reported pertain to healthy white male rabbits weighing 1600 to 1800 gm. A survey was first made to locate readily available lymph nodes adjacent to a good skin surface. The skin over the right mandible and the nearby right deep cervical lymph

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node were selected for study as being ideal because of convenience and consistency of lymph node size.

As a control study, to obtain baseline figures, right and left deep cervical lymph nodes of 5 normal rabbits were removed and divided. One half was immediately fixed for a paraffin section and the cut surface of the other half was carefully scraped with a sharp knife so that the scrapings floated into normal rabbit serum. In this way the entire contents of one-half of the node could be suspended in the serum for the preparation of smears which were quickly dried and stained by Wright's method, using buffered water of pH 6.9. Evenly distributed cells were always obtained and upon these preparations differential cell counts could be performed easily and rapidly. This method gave permanent mounts which could be restudied at any time, and for this study seemed more satisfactory than the supravital technics.[23,24] One thousand cells were counted on each side, thus giving the observer a proper survey of cell types. Slight changes in numbers of cell types could readily be recognized. Skin sections, lymph node sections, and peripheral blood smears were obtained also.

Figure 1 illustrates the six cell types which may be seen in the lymph node smears. Table I shows the distribution of these cells per 1000 and includes disintegrated cell elements. Heterophil granulocytes are very scarce in the normal lymph node smears.

It seemed advisable to study peripheral blood smears for possible reflected changes in cell types. Table II shows the results of differential and total blood counts of 11 normal rabbits. These counts correspond quite well with the figures published by those who have done hundreds of counts.[25,26] Emphasis was placed upon the differentiation of large and small lymphocytes so that any alteration might be correlated with lymph node smears.

Changes in Skin, Lymph Nodes, and Blood of Rabbits Injected with Antigen (Eberthella typhosa)

Rabbits were injected intradermally over the right mandible with 0.5 cc. of killed *Eberthella typhosa* bacilli in concentrations given below. A single dose was given to all at the same time and then the animals were killed at intervals as described below. The following materials were saved for study: (1) skin for sections stained with hematoxylin and eosin, (2) lymph nodes (right and left) for hematoxylin and eosin preparations, smears, and extraction for antibody titer, (3) serum for antibody titer, and (4) blood smears for differential counts.

Experiment 1. Five rabbits received 0.5 cc. of the vaccine containing 1 billion bacilli per cc., as superficially as possible into the skin

over the angle of the right mandible. A sixth animal, as a control, received a sterile saline injection in the same location. The 5 antigen-injected rabbits were bled, blood counts were taken, and the animals were killed by air injection on the 2nd, 5th, 8th, 10th, and 15th days. During the course of the experiment none of the animals showed untoward effects of the injection and the control rabbit was sacrificed and examined on the 3rd day. All animals were autopsied immediately

TABLE I

Differential Lymph Node Cell Counts (1000 Cells) on 5 Normal Rabbits

Rabbit no., site	Small lympho-cytes	Large lympho-cytes	Basophilic lympho-cytes	Lympho-blasts	Pseudo-eosinophils	Reticulum cells	Disinte-grated cells
1. Right	736	207	48	9	0	0	0
1. Left	793	18	2	9	1	0	177*
2. Right	890	56	30	20	0	4	0
2. Left	909	55	22	12	1	0	1
3. Right	801	165	21	9	1	0	3
3. Left	770	200	15	12	2	0	1
4. Right	816	163	10	9	0	0	2
4. Left	785	157	11	14	0	0	33
5. Right	904	64	4	12	0	1	15
5. Left	859	97	14	7	1	1	22
Average	826	118	17.7	11.3	0.6	0.6	25.4

*Defective smear.

after death and the cervical lymph nodes were prepared and smeared as described. In addition the skin and subcutaneous tissue at the injection site was fixed and sections prepared.

Experiment 2. The same experiment was repeated using 0.5 cc. of antigen which had a concentration of 10 billion bacilli per cc. These rabbits were killed on the 1st, 2nd, 3rd, 5th, and 10th days, and material from them prepared in a similar manner.

Experiment 3. Another group of 5 male rabbits and a control were treated as in experiment 2 except that one-half of each lymph node was ground in sterile sand and 2 cc. of physiologic saline solution. Each portion was weighed prior to the grinding. The cloudy suspension was frozen and thawed several times and portions were used for agglutination tests. These animals were killed on the 1st, 3rd, 5th, 7th, and 10th days.

Experiment 4. A group of 7 rabbits, with 1 control, were injected as in experiment 2, but in this series the animals were killed after 6 and 12 hours, and after 1, 2, 3, 4, 7, and 12 days. The object was to observe the earliest cell changes.

The serologic studies undertaken in this group of experiments were performed to observe changes in agglutinin content of the serum and

the lymph nodes and to correlate these changes with cellular changes. In the first three groups of animals serologic tests were carried out using the usual tube dilution technic. Maximum agglutination occurred using 5 billion bacilli per cc. In experiment 4, Heidelberger and Kabat's [27] agglutination and agglutinin nitrogen method was used.

Results

Skin

A uniform progressive change occurred in the skin of all of the antigen-injected animals, and this response may be described in the following phases extending from 6 hours to 15 days: *Phase 1*, a diffuse

Table II

Total and Differential (per 1000 cells) Peripheral White Blood Cell Counts on Normal Rabbits

Rabbit no.	Total white blood cells	Small lympho-cytes	Large lympho-cytes	Pseudo-eosino-phils	Neutro-phils	Mono-cytes	Baso-phils	Eosino-phils
1	8,650	13	26	46	1	3	8	3
2	5,900	27	20	49	1	1	2	0
3	7,200	26	28	42	0	2	2	0
4	8,000	40	16	40	0	2	2	0
5	9,400	32	23	41	0	0	4	0
6	4,500	44	9	35	2	3	6	1
7	9,800	32	23	45	0	0	0	0
8	12,800	40	12	45	0	0	2	1
9	8,750	23	17	57	0	0	2	1
10	5,500	18	17	60	0	2	2	1
11	7,600	31	20	41	0	4	3	1
Average	8,009	29.6	19.2	45.6	0.36	1.5	3.0	0.73

edema and infiltration of the upper corium with pseudo-eosinophilic and eosinophilic granular leukocytes and no trace of the injected bacilli in bacteria-stained sections. The duration of this change was 48 hours (Figs. 2 and 3). *Phase 2*, heavy concentrations of these leukocytes and focal collections in the deeper portions of the corium and in the superficial muscle layer. Infrequent large macrophages were present. The duration of this change was 3 to 6 days. *Phase 3*, return of the skin to normal except for one or two pinhead-sized yellow areas in the corium which on microscopic examination had a tiny, central, necrotic zone and a granulomatous capsule of macrophages and fibroblasts. Healing had all but been completed. The duration was 8 to 15 days.

Lymph Node Smears

Certain distinct changes in numbers and proportions of cells occurred in the lymph nodes during the course of the experiments. Table III presents the results of differential counts done on the right cer-

vical lymph nodes of all rabbits in the four experiments. This comparison is allowable since the animals received the same antigen under the same conditions (excepting the smaller dose in experiment 1).

The figures in Table III which definitely appear to deviate from the normal have been printed in italicized type. From the lowest horizontal row of figures the normal lymph node counts can be determined.

TABLE III

Comparison of Time Interval and Differential Lymph Node Count (All Four Experiments Represented)

Time	Experiment	Small lymphocytes	Large lymphocytes	Basophilic lymphocytes	Lymphoblasts	Disintegrated cells	Reticulum cells	Pseudo-eosinophils	Serum titer
6 hr.	4	740	140	9	12	8	0	*91*	0
12 hr.	4	804	92	8	10	17	0	*69*	0
24 hr.	3*	818	107	19	7	15	0	*34*	0
2 days	1	798	108	34	*55*	0	0	5	0
2 days	2	634	286	8	*53*	7	7	5	0
2 days	4	713	243	8	*28*	7	0	1	0
3 days	2	682	209	9	*96*	0	2	2	0
3 days	3	853	84	4	*57*	0	0	2	0
4 days	4	697	200	5	*6*	91	0	1	†
5 days	1	744	157	*32*	*53*	11	0	3	0
5 days	2	712	200	*69*	17	2	0	0	1:160
5 days	3	803	139	*17*	26	14	0	1	1:20
7 days	3	676	226	*52*	31	13	0	2	1:80
7 days	4	799	166	8	17	14	1	5	
8 days	1	785	196	8	9	0	0	5	1:40
10 days	2	645	*302*	14	15	14	0	10	1:160
10 days	3	657	*301*	8	15	17	0	2	1:640
11 days	1	816	162	10	9	2	1	0	1:40
12 days									
15 days	1	900	64	4	12	15	1	4	1:20
Normal average (Table I)		826	118	17.7	11.3	25.4	0.6	0.6	

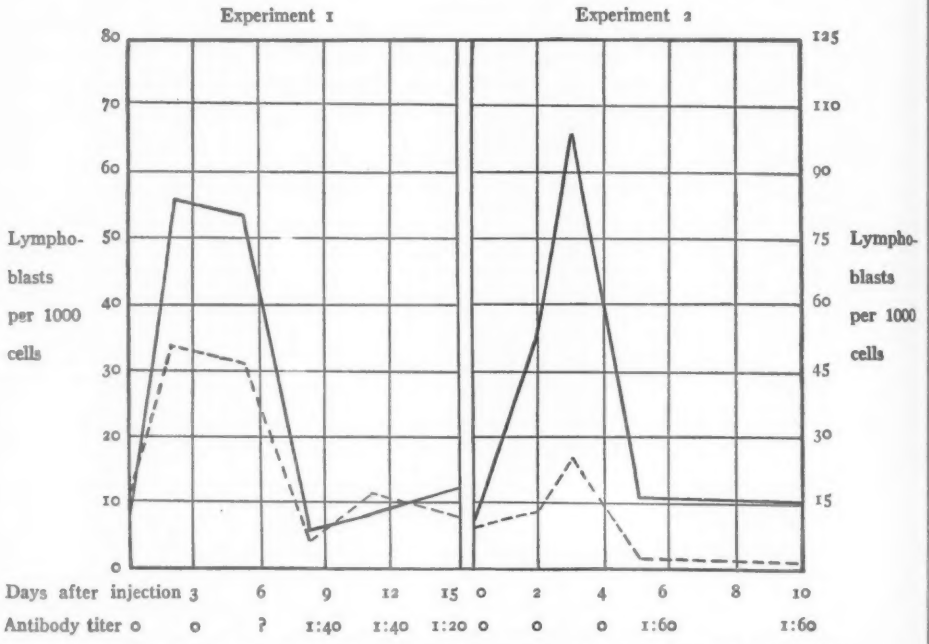
* Slides lost on 24-hour rabbit, experiment 4.

† See serologic discussion.

Ranges of normal are within a few cells in each case, with the exception of the large lymphocytes which rarely rise to 200 cells per 1000. The first abnormal change to occur is the sudden rise of pseudo-eosinophilic granular leukocytes and eosinophilic leukocytes. These two cells have not been separated in this study because there seems to be no clear-cut differentiation. Cells of the pseudo-eosinophilic type are filled with large red granules, and one can only say that the true eosinophils appear to be packed with these same granules (Figs. 2 and 3). One gains the impression from examining many of these slides that in the non-injected animals the pseudo-eosinophils have small, well scattered, red granules while in the recently injected rabbit these cells take on the appearance of true eosinophils. However this may be, these cells are greatly increased in number at the first observation interval of 6 hours

and decrease rather rapidly until at 48 hours their number is reduced to normal.

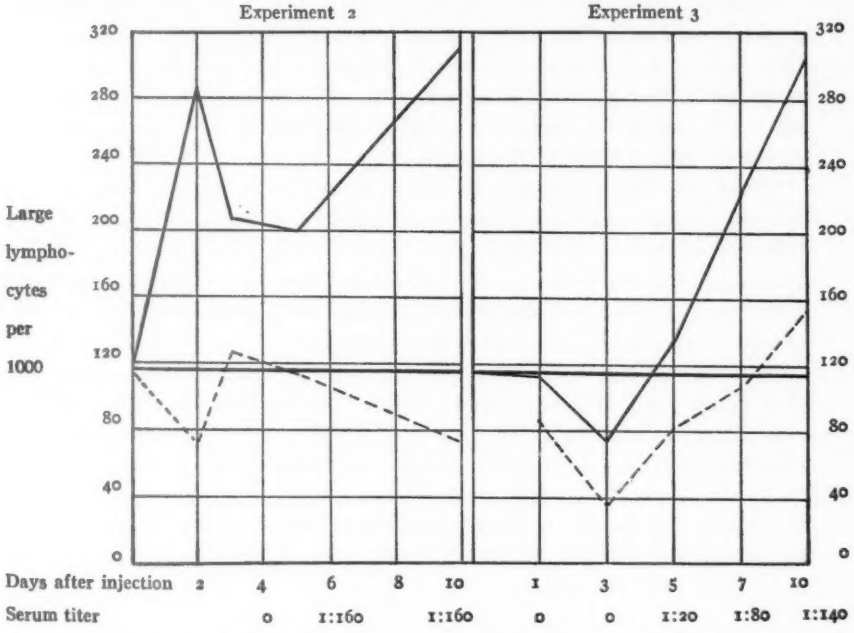
At the time that the eosinophilic cells decrease (48 hours), the lymphoblasts go through an interval of hyperplasia which lasts for 3 days or more, during which there is a five-fold increase in their number. Text-Figure 1, which compares the numerical changes in lympho-



Text-Figure 1. Lymph node differential counts. The curves show the sudden rise and fall of lymphoblasts between the second and sixth days after injection. The solid line indicates results on smears from the right nodes, and broken line, from the left nodes.

blasts with the time interval in experiments 1 and 2, shows this diagramatically. The diagram includes the differential counts done on the left cervical nodes and shows that a slight simultaneous hyperplasia of lymphoblasts is present in these nodes too. Table III shows that when the experiment is repeated several times the number of lymphoblasts decreases gradually over 4 to 6 days, while Text-Figure 1 shows a precipitous drop in lymphoblasts in each experiment. It must be kept in mind that these experiments include only a 15-day interval, which might be referred to as the primary phase of cellular changes in the rabbit.

Basophilic lymphocytes, which have been compared to the plasma cell, share in the hyperplasia with the lymphoblasts but at a slightly later interval. During the first two experiments it was thought that this basophilic cell increase was an isolated observation, but the aggregate of experiments shows a small but definite band of hyperplasia from the 5th to the 7th day just at the time of appearance of an agglu-



Text-Figure 2. Lymph node differential counts. The solid line indicates the increased number of large lymphocytes in smears from the right nodes after lymphoblastic hyperplasia. The broken line shows the number of large lymphocytes in smears from the left nodes.

tinin titer. This is not to be interpreted as a definite correlation at this time.

The evaluation of the changes in number of the large lymphocytes is somewhat more difficult but it may be stated generally that there appears to be a general increase after the interval of lymphoblastic activity. In order to bring this out it is necessary to examine Text-Figure 2, which shows the occurrence of these cells in experiments 2 and 3. Especially in experiment 3 the right lymph node smears show a steady rise in the number of large lymphocytes. The heavy black line across the center of the figure indicates the normal number of large lymphocytes per 1000 cells. It is observed also that it is these cells

which pinch off small globules of blue-staining cytoplasmic material, which has been referred to as "shedding."[28] It seems to me that wherever there are large lymphocytes this shedding phenomenon occurs, no matter what interval of antigen stimulation is represented. More shedding, of course, occurs when the number of large lymphocytes increases.

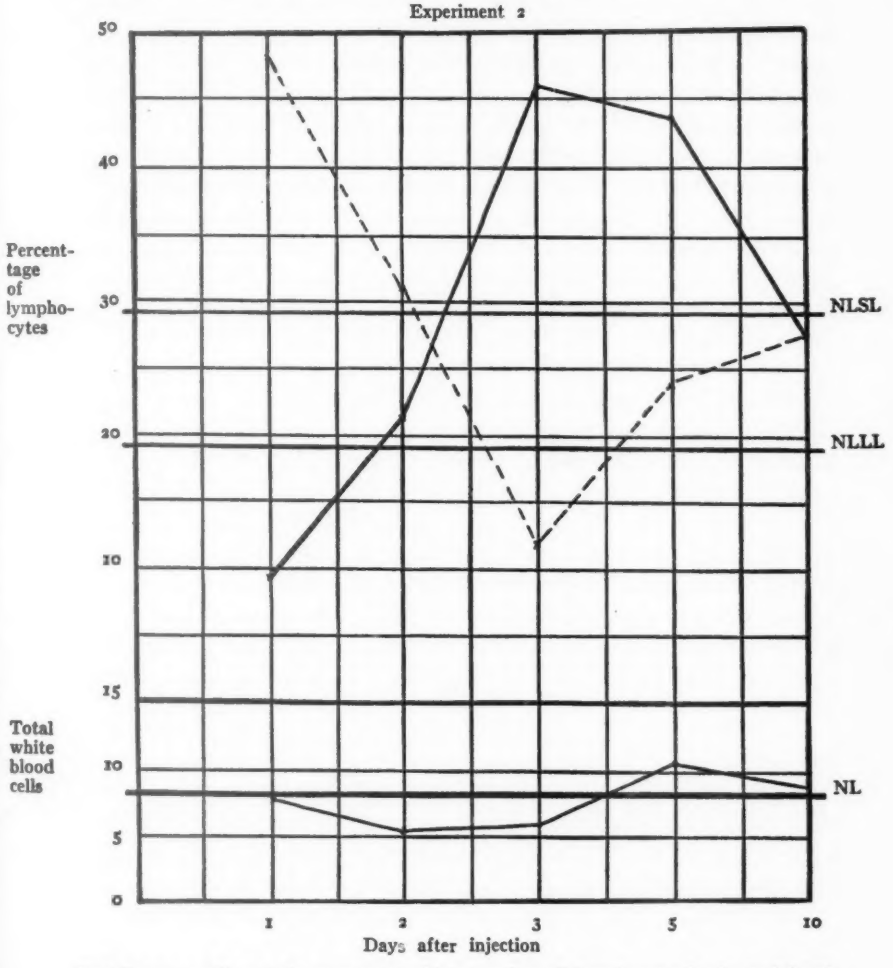
Another observation must be included for whatever interpretation might be deduced. Frequently, large lymphocytes were found to contain eosinophilic granules in their cytoplasm, not unlike those of eosinophilic leukocytes. This observation was made only at the stage when the eosinophils were disappearing (48 hours), and many granules were found loose in the smear.

Other cells differentiated in the nodal smears did not go through significant changes. Small lymphocytes are always present in large numbers and, of course, their number decreases as other cells become hyperplastic. But this does not measure the true status of this group since a fixed number of cells is counted. Comment on this point will be included in the section on the fixed lymph node material. Reticulum cells failed to show hyperplasia during the time interval represented. One would think that had this cell been stimulated to perform some function, a slight or moderate change in number would have occurred. Disintegrated cells seemed to increase somewhat during the longer intervals. Since these cells seemed to represent broken-down large lymphocytes with only the nuclear portion remaining, this increase is understandable because of the increase in large lymphocytes in the more prolonged experiments.

Lymph Node Sections

Cellular changes in the sections of lymph nodes were inconspicuous when compared to the changes in the smears. Edema and hyperemia of the hilar region and medullary zone (Figs. 4 and 5) occurred early in the right cervical nodes but not in the left. This was followed and accompanied by an infiltration of pseudo-eosinophilic leukocytes in the same areas. These leukocytes seemed to gather in groups of about 6 or 8 cells and were rarely found in the cortical sinuses. Some appeared to be undergoing disintegration, as was noted in the smears, but in the sections a few large cells with distinctly smooth, eosinophilic cytoplasm were usually nearby (Fig. 5). As seen under the oil immersion lens, they could have been either reticulum cells or large lymphocytes. One day later, at 72 hours, lymphoblastic activity was evident, scattered throughout the medullary zone and not, as might be expected, in the

primary follicles. On close inspection one could not make out any significant changes in the primary follicles despite the rather obvious activity going on in the central portion of the lymph node. There was a possible increase in small lymphocytes in the sinusoids. By the 5th day the lymph node sections appeared normal and the appearance was then identical to that of the left cervical nodes.



Text-Figure 3. Changes in percentage of large and small lymphocytes in the peripheral blood in antigen-injected rabbits. The upper portion shows the reversal of small lymphocytes and large lymphocytes after lymphoblastic hyperplasia. The lower portion shows essentially no change in total white count. NLSL, normal line of small lymphocytes. NLLL, normal line of large lymphocytes. Broken line, small lymphocytes; solid line, large lymphocytes.

Peripheral Blood Smears

At the time each animal was autopsied a total white and differential blood count was performed. Text-Figure 3 shows the significant changes, which appeared to be limited to the lymphocytic series for the most part. At the lower portion of this figure is shown the total white cell count, which did not deviate significantly from the normal. Above is shown the reciprocal behavior of the large and small lymphocytes during the course of the experiment. The change in preponderance from small to large lymphocytes is seen to occur at the interval of the increase of large lymphocytes in the lymph node smears. This seems to show then that these large lymphocytes in the nodes get into the general circulation rather quickly. A slight relative lymphocytosis occurred during the period of the reversal of small and large lymphocytes. Very little change in the number of cells of the granular series could be observed despite the rather intense leukocytic infiltration in the skin at the site of the injection of the antigen. The nature of the eosinophilic leukocytic change is to be studied in more detail in other experiments.

Serologic Studies

Two sources of material, serum and ground-up lymph node in saline solution, were utilized in performing agglutination tests. The lymph node suspension was made by grinding a weighed portion of cervical node in 3 cc. of physiologic saline solution and sand, decanting off the cloudy fluid, and finally freezing and thawing the mixture three times. The sediment was then smeared to be sure that no intact cells remained, after which the material was centrifuged for 2 minutes at 1500 r.p.m. and the clear fluid separated for the tests. By this technic the number of mg. of lymph node material per cc. of physiologic saline solution can be determined readily. Table IV gives the agglutination

TABLE IV

Serum and Lymph Node Titers of Rabbits of Experiment 3
Antigen, 5 Billion Organisms per 1 cc.

	Rabbit 2, 1st day		Rabbit 3, 3rd day		Rabbit 4, 5th day		Rabbit 5, 7th day		Rabbit 6, 10th day	
Titer	Serum	Node	Serum	Node	Serum	Node	Serum	Node	Serum	Node*
1:2	−	−	−	−	+	−	++	++	+++	++
1:4	−	−	−	−	+	−	+++	+	++	+
1:20	−	−	−	−	+	−	++	−	+++	−
1:40	−	−	−	−	−	−	+++	−	++	−
1:80	−	−	−	−	−	−	++	−	++	−
1:160	−	−	−	−	−	−	++	−	++	−
1:320	−	−	−	−	−	−	+	−	+	−
1:640	−	−	−	−	−	−	−	−	−	−

*Nodal suspensions equal 20 to 30 mg. of material per cc.
Serum represents 60 to 70 mg. of protein per cc.

results by the usual tube dilution method applied to the serum and lymph node suspensions in experiment 3.

Calculation of the nitrogen content of the serum and the nodal suspensions showed that the serum contained fifteen times as much protein, but in spite of this correction the serum far outstripped the nodal material in its ability to agglutinate the antigen. Heidelberger and Kabat's [27] method was attempted in experiment 4, but the results will not be given because I could not be sure of their accuracy without the use of a constant temperature cold centrifuge. It is not the purpose of this report to contest the serologic aspects of serum versus lymph node antibody content, but the data collected so far do not agree with previous reports.[13,18] It is believed that, given the proper equipment, the Heidelberger-Kabat method is probably more accurate than tube dilution methods.

Summary

A method has been developed for performing differential cell counts on lymph nodes of rabbits. Differential counts of lymph nodes of normal rabbits were first determined, which gave baseline figures.

The following observations were made upon the skin and lymph nodes of rabbits injected cutaneously with *Eberthella typhosa* as antigen:

1. The bacteria disappeared from the corium within 6 hours and a diffuse pseudo-eosinophilic and eosinophilic infiltration took place which lasted for 48 hours.
2. During this same interval eosinophilic granulocytes appeared in the regional lymph node in large numbers and seemed to disintegrate there.
3. In the interval of 2 to 5 days a small focal granuloma formed in the skin at the site of injection and lymphoblasts became distinctly hyperplastic in the lymph nodes.
4. During the 5 to 7 day period basophilic lymphocytes increased moderately in number in the lymph nodes and the serum antibody titer started to rise.
5. From this time until the end of the experiments at 15 days, large lymphocytes usually were increased in number both in the nodal smears and in the peripheral blood smears. At this time the skin lesion was only a minute focus of fibroblasts and macrophages.

It may be concluded from these changes that from the standpoint of cellular pathology it appears that antibody formation in this primary phase is quite complicated and that it is in all probability a pluricellular reaction involving several systems of cell types.

It is deduced that granulocytes, lymphoblasts, large lymphocytes,

and possibly basophilic lymphocytes (plasma cells) play an important part in the primary phase of antibody formation.

I wish to acknowledge the many helpful suggestions made by Dr. F. D. Gunn.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 209

All plates are drawings of microscopic fields by Keith Eddington.

FIG. 1. Reproduced from colored drawing illustrating the six classified cell types from lymph node smears.

A. Pseudo-eosinophilic granular leukocyte which is rarely seen in the normal smear.
B. Lymphoblast with nucleoli.
C. Basophilic lymphocyte.
D. Large lymphocyte.
E. Small lymphocyte.
F. Reticulum cell.

See text for discussion and differential counts.

A

M

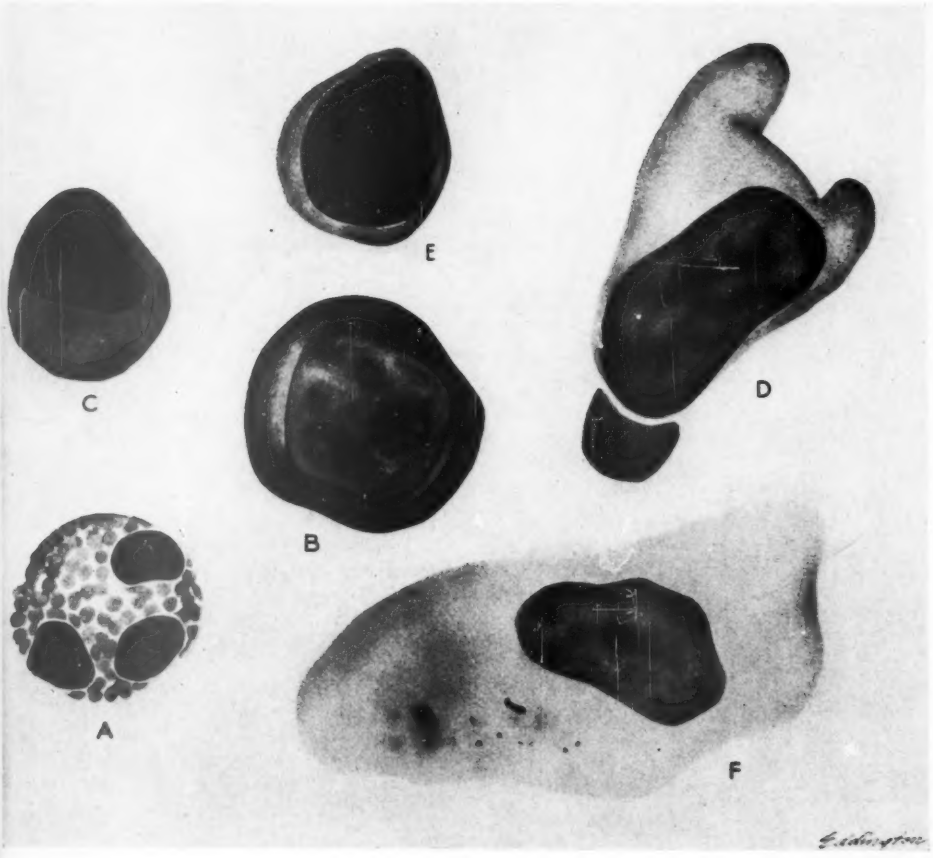

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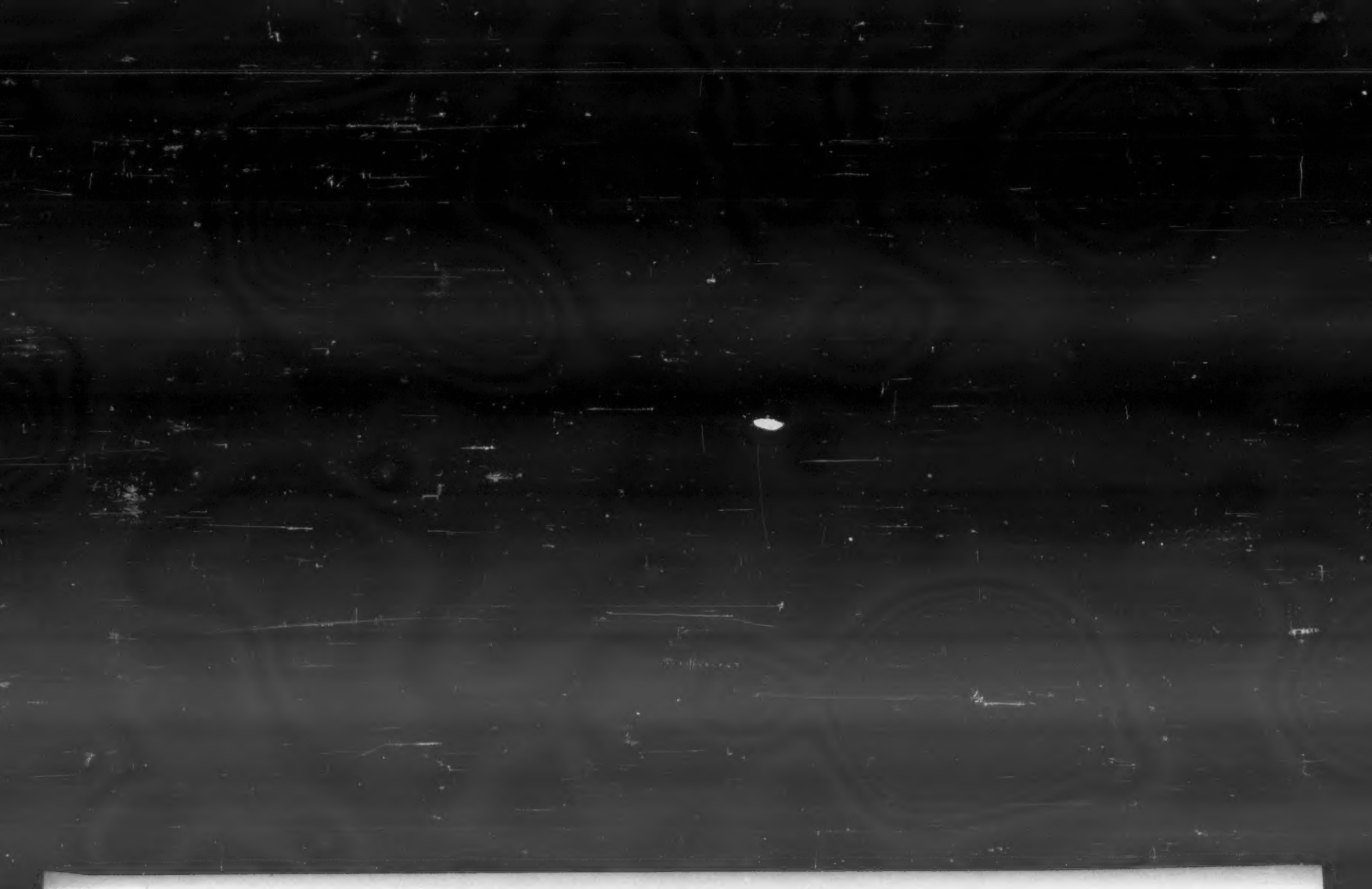
PLATE 210

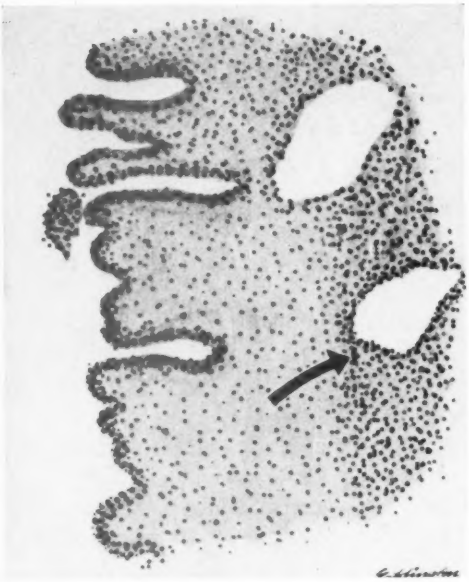
FIG. 2. There is a diffuse granular leukocytic infiltration of the corium. Lymphatics are dilated and cellular elements crowd these spaces. Time interval after antigen injection, 12 hours. Hematoxylin and eosin stain. × 100.

FIG. 3. High-power drawing of cell types seen at site of arrow in Figure 2. Capillaries are dilated and pseudo-eosinophils (A) are numerous. A fair number of true eosinophils (C) and rare neutrophils (B) are present. No bacteria are seen. Hematoxylin and eosin stain. × 1000.

FIG. 4. Low-power view of central zone of right cervical lymph node 24 hours after injection. Many areas of hyperemia (A) are seen and also dilated clear spaces (B) which appear to be lymphatic channels, some of which contain lymphocytes. Hematoxylin and eosin stain. × 100.

FIG. 5. High-power view illustrates cell types seen in right lymph node at 24 hours before lymphoblastic activity commences. Pseudo-eosinophils and eosinophils (A) appear in sinuses and a few large lymphocytes (B) are nearby. Some of the eosinophilic cells are disintegrating (C). Endothelial cells (D) and reticulum cells appear undisturbed. Small lymphocytes are scattered about.





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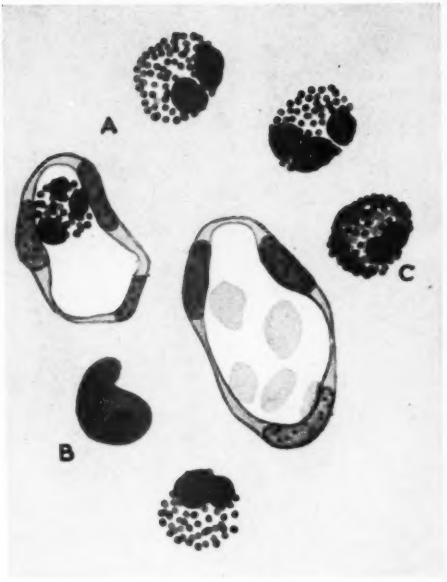

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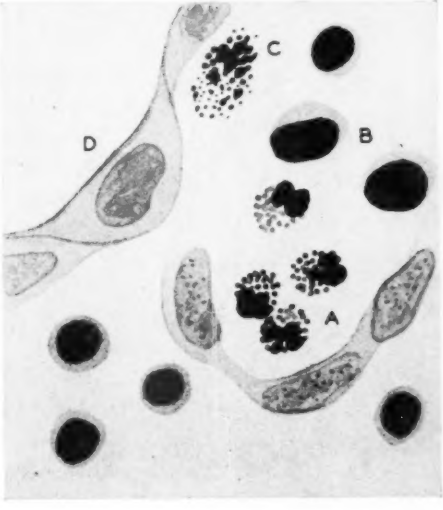

5

THROMBOTIC (EMBOLIC) AORTIC OCCLUSION
Its Occurrence Among Mentally Sick Patients *

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Occluding emboli and/or thrombi of the aorta are considered to be rare. In general hospitals they are found in about 0.12 per cent of all autopsies.[1-5] In the autopsy material of mental hospitals small aortic thrombi are a common finding in elderly patients, and obliterating aortic thrombi are not rare. An explanation was sought for the high incidence of aortic thrombi among mentally sick patients.

Material

During a 4-year period, 382 autopsies were performed on psychotic patients. Aortic thrombi of varying size were found in 57 cases (18 males and 39 females). Psychoses of all types were responsible for the admission of these patients to the institution, but senile and arteriosclerotic psychoses occurred in 50 per cent of the cases. According to the size and the nature of the thrombi, the 57 cases were divided into three groups. The largest group (I) consisted of 37 cases with small adherent thrombi.† The smallest group (II) comprised 7 cases of aortic aneurysm, 6 of which were of arteriosclerotic origin and showed thrombi inside and outside the aneurysm. Group III consisted of 13 cases with large aortic thrombi which obliterated the lumen of the vessel.

The fatal disease varied. Bronchopneumonia, pulmonary embolism, myocardial infarction, coronary arteriosclerosis and thrombosis, cerebral thrombosis and hemorrhage, and severe enterocolitis were the main causes of death. Wasting diseases such as cancer or caseous tuberculosis were rare. The nutritional condition and the ages of the patients can be seen in Tables I and II.

Group I

Small adherent thrombi were found in the aortas of the 13 men and 24 women comprising group I. Nutritional condition and age are shown in Tables I and II, respectively. The psychoses for which the patients had been treated were of different types (mental deficiency, schizophrenia, paranoid state), but arteriosclerotic psychoses were predominant. The thrombi were found over or near atheromatous plaques and they were always situated in the abdominal aorta near the bifurca-

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† After conclusion of the study, 10 more cases (5 men and 5 women in the age between 65 and 96) showed small nonobliterating thrombi of the aorta.

tion. In 14 cases thrombi were found also in the descending thoracic aorta, and in 8 cases in the arch and the ascending aorta. None of these thrombi had occluded the lumen. The fatal disease varied; bronchopneumonia was the most common cause of death. In addition to atheromatosis of the aorta, sclerosis of the heart valves, and arteriosclerosis of the coronary, renal, and cerebral arteries were usually present.

Group II

Aortic aneurysm was relatively rare in this material. Only one large syphilitic aneurysm of the ascending aorta was observed. This was found in a 57-year-old man, hospitalized for 30 years with a diagnosis

TABLE I

Distribution of Cases as to Nutritional State

	Total	Poor	Moderate	Obese
Total	57	28	12	17
Group I	37	18	9	10
Group II	7	4	2	1
Group III	13	6	1	6

TABLE II

Distribution of Cases According to Age

	31–40 years	41–50 years	51–60 years	61–70 years	71–80 years	81–90 years	Over 90 years	Total
Total	1		5	8	21	20	2	57
Group I			2	4	15	15	1	37
Group II			1		3	2	1	7
Group III	1		2	4	3	3		13

of dementia praecox, paranoid type. Arteriosclerotic aneurysms were seen in 6 female patients, 71 to 93 years old. Only one patient was obese and died suddenly from a rupture of a large fusiform aneurysm of the descending aorta.[6] The other 5 patients were poorly or moderately nourished and had saccular aneurysms of the abdominal aorta. Adherent blood clots filled the aneurysm and were seen also in other parts of the aorta, over atheromatous plaques. One patient had a second aneurysm of the descending aorta. None of these saccular aneurysms had ruptured. All 6 female patients had been diagnosed as having arteriosclerotic or senile psychosis. In one there was cachexia due to carcinoma of the breast with metastases.

Group III

The third group of obliterating aortic thrombi consisted of 4 men and 9 women. It can be seen from Table I that poorly nourished and obese patients were equal in number. Only one patient was younger

than 50 years. She was a well nourished mentally deficient woman, 33 years old. The cause of her aortic thrombosis was polycythemia. The same underlying pathologic condition was responsible for the thrombosis found in 2 other women, 72 and 80 years old. In the 72-year-old woman the thrombi were situated in the arch of the aorta and had extended into the right innominate, subclavian, and carotid arteries, causing gangrene of the right arm. In the other 12 cases the thrombi were situated in the abdominal aorta and extended upward from the bifurcation over a varying area and downward into one or both iliac arteries and their branches. In 4 cases the orifice of one renal artery was more or less occluded by the thrombus, resulting in large infarcts and atrophy of the kidney. In one case the orifices of both mesenteric arteries were occluded and terminal intestinal gangrene was found. Total obliteration of one or both iliac arteries had caused gangrene of one or both legs shortly before death in 9 instances. The fresh obliterating thrombi were soft and dark. In 4 cases, long-standing thrombi, silent for an undetermined period, were found firmly attached to the aortic wall, appearing as mushy, grayish yellow clots. Microscopically, these thrombi were homogenously pale-staining with only a few cellular elements in them. A fibrinoid layer separated them from the proliferated intima. There were a number of histiocytes with hemosiderin in the intima and numerous round-celled accumulations in the intima, media, and especially in the adventitia, indicating a reaction to thrombosis. Patients with senile and arteriosclerotic psychoses, mental deficiencies, schizophrenias, and alcoholic psychoses were found in this group.

From the presence of mural thrombi in the distended left auricles of 2 women with chronic mitral valvulitis and mitral stenosis, it was concluded that the obstruction was primarily embolic, followed by secondary thrombosis. One was a 55-year-old schizophrenic, the other a 68-year-old arteriosclerotic patient. An embolic origin appeared possible in 2 cases with coronary occlusion, myocardial infarction, and mural thrombi in the left ventricle and in one case with a distended left auricle containing mural thrombi, and with coronary and generalized arteriosclerosis. However, formation of the clot by thrombosis could not be excluded. In 8 other cases without mural thrombi in the heart, primary thrombosis at the bifurcation was probable. Included are the 3 cases of polycythemia in which an increased tendency to clotting existed during life. In the remaining cases atheromatosis of the aorta and impairment of the blood flow by coronary sclerosis, myocardial infarction, or calcific valvulitis were the main reasons for the formation of the thrombus. Ten of the 13 patients were over 60 years of age and showed arteriosclerotic heart disease and/or generalized arterioscle-

rosis. Atrophy of the organs was common, as is usual in elderly psychotic patients.[7] Weight of the brain varied from 950 to 1050 gm.; of the spleen, from 50 to 100 gm.; of the liver, from 860 to 1050 gm.; and of one kidney, from 50 to 100 gm. The heart showed usually a moderate hypertrophy with an average weight of about 400 gm. In only a few cases, as in the patients with polycythemia or in very obese patients, was there encountered a brain weighing 1400 gm., a spleen weighing 250 gm., a kidney weighing 210 gm., a liver weighing 1880 gm., or a heart weighing 600 gm.

One of the cases was unusual because the silent thrombosis extended from the isthmus of the aorta to the bifurcation, occluding the left renal artery and extending into both iliac arteries. The patient, a 72-year old, well-nourished woman with an alcoholic psychosis, had been hospitalized for many years and had been treated for diabetes. Signs of the thrombosis developed only 2 days before death when the right iliac artery became wholly obstructed and gangrene of the right foot developed. Thrombosis of the left renal artery had resulted in total necrosis of that kidney with superimposed suppuration, but no evidences of renal damage had been observed during life. The gross and microscopic picture of the necrotic kidney was different from that of necrotizing papillitis observed in diabetic patients.[8-10]

Discussion

It is estimated by most observers that aortic occlusions of embolic or thrombotic origin are found in only about 0.12 per cent of all autopsies performed in general hospitals.[1-5] Obliterating aortic thrombi have been observed at every age, even in infants.[11] The diagnosis has often been made during life; recently several such cases have been reported,[2,12] The thrombus has been removed by surgical approach successfully in a number of instances.[13-20] Popkins' opinion [21,22] that aortic occlusions occur more often than is generally believed was confirmed by the autopsy material presented in this study. Some of the occlusions occurring in mentally sick patients were diagnosed before death; more were discovered incidentally at necropsy. It is believed that in the younger age group the obliteration of the aorta is more often of embolic origin, associated with mitral stenosis, auricular fibrillation, and mural thrombi in the left auricle.[1-3] Only 2 cases of mitral stenosis were found in my material. Although 382 autopsies is a relatively small group from which to draw statistical conclusions, the fact remains that the percentage of aortic thrombi was much higher than has been reported by other observers. Small adherent thrombi over atheromatous ulcers of the aorta were a common finding in psychotic patients over 60 years of age, and were observed in nearly 15 per

cent of all autopsies (57 of 382 autopsies). Obliterating thrombi were found in 3.4 per cent of the autopsies (13 of 382 autopsies). Neither the type of psychosis, which varied to a large degree, nor the nutritional condition was an important factor. Emaciated and obese patients showed the same tendency to the formation of small mural or obliterating aortic thrombi. It may be that some of the patients were better nourished before the onset of their mental or physical disease, but a number of the emaciated patients had been hospitalized for a long period and no chronic wasting diseases were found to be responsible for the emaciation. Mental disturbances, the difficulty in feeding, the slowing of metabolism, and the generalized arteriosclerosis explained the poor nutritional state of the patients.[7] The fatal terminal disease was too variable to account for the frequency of aortic thrombi. This frequency is probably due to the fact that 254 of 382 autopsies, or 66 per cent, were performed on patients over 60 years of age and that 160 of 382 autopsies, or 42 per cent, were performed on patients over 70 years of age, in whom more or less advanced atherosclerosis, especially in the abdominal aorta, was present. Elderly patients form a large proportion of the inmates of mental hospitals at the present time, and it is probable that the number of these patients will increase with the ageing of the whole population. Although obliterating thrombi may remain silent for some time, it seems likely that some of the thrombi were found incidentally at necropsy only because the confused and mentally deteriorated patients did not complain before stormy symptoms and gangrene had developed. Many of these patients had been admitted to the hospital because of mental symptoms which were due to impairment of the cerebral circulation and to cerebral arteriosclerosis. It is understandable that these patients often showed other vascular disturbances on the same basis, such as coronary disease, myocardial infarctions, calcific valvulitis, aortic atheromatosis, and aortic thrombi.

Summary

Small aortic thrombi in atheromatosis of the aorta are frequent in mentally sick patients over 60 years of age. Thrombotic aortic occlusions among this special group are not as rare as would be concluded from the autopsy material of general hospitals. In the material utilized for this study, aortic thrombosis was found in 57 of a series of 382 autopsies. In 13 cases the thrombus was obturating. The reason for the greater frequency in the mentally sick group is, apparently, the more advanced average age of this group.

Since submitting this paper for publication, 6 more cases of aortic thrombosis have been observed. The patients were from 71 to 91 years old and had signs of advanced sclerosis of the aorta, coronary arteries, and heart valves. In 2, gan-

grene of the legs, and in one, gangrene of the intestines, had developed shortly before death. In one instance the thrombus was situated at the isthmus. In the other 5 cases it was found at the bifurcation and extended into the iliac arteries.

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